INFLUENCE OF PROTEIN INTAKE AND STAGE OF GESTATION ON GROWTH, REPRODUCTIVE PERFORMANCE, NITROGEN BALANCE, AND TISSUE LEVELS OF PROTEINS, DNA, AND RNA IN GILTS

By

RICHARD DON JONES II

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1969

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1973

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 1975

INFLUENCE OF PROTEIN INTAKE AND STAGE OF GESTATION ON GROWTH, REPRODUCTIVE PERFORMANCE, NITROGEN BALANCE, AND TISSUE LEVELS OF PROTEINS, DNA, AND RNA IN GILTS

Thesis Approved:

Charles V. Maxwell
Thesis Adviser

Rodger Johnson

Robert A. Wettemann

George H. Odell

Fredric N. Owens

N. N. Durham
Dean of the Graduate College

ACKNOWLEDGEMENTS

The author wishes to take this opportunity to thank Dr. C.V. Maxwell, major adviser, for guidance and support in the course of this thesis project.

Special thanks are extended to Dr. R.K. Johnson for assistance in statistical analysis of data and critical evaluation of the thesis. Appreciation is given to Dr. R.R. Johnson, Dr. George V. Odell, Dr. Fred N. Owens, and Dr. R.P. Wettemann for their suggestions and professional guidance.

Special recognition is extended to Rex Vencl, herdsman, who not only cared for the experimental animals, but also devoted particular attention to the details of the study. Others who deserve thanks are Dr. Keith Lusby, Leroy Kimbrell and numerous fellow graduate students.

This thesis is dedicated to my wife, Ruthann, and daughter Kelly because they have worked hard and continued their support during these years of graduate studies.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

CHAPTER I

INTRODUCTION

It has been shown that the gestating gilt has the capacity to retain more nitrogen in the body than can be accounted for by increases in direct products of conception. This retention increases as protein intake increases and has been attributed to storage of protein in extrauterine tissues. The extent, nature and physiological importance of such protein reserves has not been documented in the gilt.

Other questions arise as to the possible translocation of stored protein from the dam's tissues to fetal tissues at advanced stages of gestation. Is cellular growth in the gravid uterus aided by elevated dietary protein intakes or translocated storage protein?

The aims of this study were (1) to determine the effects of protein intake and stage of gestation on growth, reproductive performance and nitrogen balance in gilts and (2) to evaluate tissue growth, especially protein, DNA and RNA contents of skeletal muscle and the gravid uterus. These investigations should lead to a more detailed definition of the protein requirements of the gestating gilt.

CHAPTER II

LITERATURE REVIEW

This review of literature involves (1) the effects of protein intake and stage of gestation on nitrogen balance, (2) reproductive performance in gilts, (3) the concept of protein storage in the gravid gilt, and (4) the relationships among dietary protein and tissue levels of protein, DNA and RNA.

Nitrogen Balance

Protein Intake Levels

Early work using nitrogen balance techniques as an indicator of protein metabolism in sows and gilts has been extensively reviewed by Duncan and Lodge (1960). The consensus of the studies reviewed was that the pregnant gilt retains much more nitrogen than can be accounted for by the fetus and associated fluids and membranes.

Rippel (1967) also reviewed the subject of nitrogen balance in relation to reproductive performance of gilts. In view of his own work Rippel (1967) stated that the level of protein intake may determine the influence of the fetus on total nitrogen retained. At protein levels which support no more than 8 to 10 g of nitrogen retention per

day, fetal weight may have little or no influence on nitrogen retention. At higher intakes of protein, fetal needs for nitrogen will be met and additional extra-uterine protein deposition occurs.

Since the gravid gilt will retain nitrogen in excess of immediate needs, nitrogen balance can be criticized as a valid indicator of dietary protein requirements. Newton (1956) states that "the stage of pregnancy is a special syndrome, initiated by the products of conception, but not adjusted from moment to moment to their requirement". Therefore, pregnancy is only a part of the maternal syndrome for which the gilt's body must prepare. Therefore, nitrogen retained by the gilt may actually reflect total requirements for maintenance, gestation, lactation and rebreeding. On this basis, retention may be considered a valid parameter of longterm dietary protein sufficiency.

Rippel et al. (1965b) studied nitrogen balance in the last one-third of gestation in gilts fed from 0 to 273 g of protein daily. These authors fitted a broken line to their data and suggested that 230 g of protein daily was sufficient to meet the gilt's needs during this part of pregnancy. But nitrogen retention in their experiment continued to increase with increased protein intakes up to their 273 g maximum.

Miller et al. (1969) also investigated nitrogen retention in late gestation (day 95) of gilts. Regression of nitrogen retained on dietary protein intakes from 114 to 342 g per day resulted in significant linear (but not quadratic)

effects. Retention did not plateau at the higher levels of protein. These authors suggested that a high ratio of essential to non-essential amino acids in the diet may reduce the protein required to maximize retention.

Work by Jones and Maxwell (1974) established that nitrogen retention of the gilt, during early pregnancy, is a linear ($P<.05$) function of protein intakes between 143 and 345 g of crude protein per day. Diets with 8 and 14 percent crude protein were produced by extending a 20 percent crude protein diet with cornstarch. This resulted in similar amino acid ratios across all diets and a high ratio of essential to non-essential amino acids in all three diets.

In conclusion, based on nitrogen balance studies in early or late gestation, retention of nitrogen by pregnant gilts increases as intake of a high quality protein mixture increases. This linear relationship extends to at least 345 g of crude protein intake daily.

Jones (1973) recently reviewed the literature on the effects of protein quantity, quality and amino acid supplementation on nitrogen balance of gestating gilts.

Stage of Gestation

The effects of stage of gestation on nitrogen balance in swine have been noted in early literature when Evans (1929) reported no increase in nitrogen retention as pregnancy progressed. However, the author did note an increased retention almost from conception indicating that the state

of pregnancy involved a changed status of nitrogen metabolism. Mitchell et al. (1931) concurred with the observation of Evans (1929) that nitrogen retention did not increase as pregnancy progressed.

More recent studies by Salmon-Legagneur and Rerat (1962) and Elsley et al. (1966) indicate that there is an early pregnancy anabolism in the gilt, possibly mediated by a change in endocrine balance, which subsides as pregnancy progresses and fetal needs increase. Campbell et al. (1953) previously suggested that the pregnant rat retains nitrogen in early pregnancy for later translocation to meet the needs of the developing fetuses.

Contrary to early studies, Elsley et al. (1966) showed a significant linear increase in the nitrogen retained by gilts as pregnancy progressed from day 1 through day 110. These data do not support the idea that the intrauterine nitrogen deposition of late pregnancy causes an exponential increase in nitrogen retention at that time as suggested by Moustgaard (1962). The work of Kline, Anderson and Melampy (1972) concurs with Elsley et al. (1966) in that nitrogen retained by pregnant gilts increased with each successive stage of gestation.

In an attempt to discover an endocrine basis for nitrogen retention during gestation, Shearer et al. (1971) studied nitrogen balance from pre-puberty through parturition. Throughout early gestation, retention remained rather constant (73.5 mg/kg body weight) then rose to 90.0 mg/kg by

day 90 and remained constant to day 110. Attempts to correlate plasma progesterone levels and retention were unsuccessful, indicating that if a relationship exists between these two measurements, it is complex.

Reproductive Performance

The topic of reproductive performance in gilts has been reviewed by Jones (1973) with general conclusions that it is very difficult to establish any direct effect of dietary protein intake on reproductive performance as evaluated by litter size or birthweight. Jones and Maxwell (1974) reported a linear ($P<.05$) increase in number of corpora lutea as protein intake increased from 143 to 345 g/day. However, number of live embryos and embryo survival rate at 30 days of gestation were not affected by protein intake.

Studies involving the feeding of fortified milo diets to gestating gilts and sows have shown little effect on reproductive performance (Calvez *et al.*, 1973; Hines *et al.*, 1973). Hesby *et al.* (1973) fed gilts different levels of protein at 24 or 84 hour intervals and found no differences in offspring performance except total litter and pig birth weights. A similar study by Haught *et al.* (1974) showed that fortified sorghum diets fed to day 80 of gestation or 12 and 14% crude protein diets were adequate when 16 percent crude protein lactation diets were fed. Thus reproductive performance criteria do not appear to respond to wide ranges of dietary protein quantity or quality.

The Concept of Protein Storage

Storage protein in the adult animal was reviewed by Kosterlitz and Campbell (1945) with particular attention to the nature and physiological significance of protein reserves. These authors defined two types of protein stores in the adult animal. The first type involves nitrogen which is retained or lost according to changes in dietary protein intake and is attributable primarily to labile liver cytoplasm and to a lesser extent, other body tissues. The second type of storage protein is depleted only under more drastic conditions such as extended fasting, protein free diets or extensive blood losses. This second type of storage is primarily muscular nitrogen and relatively extensive losses are compatable with life. These storage proteins are not likely different from common cytoplasmic proteins.

Allison and Wannemacher (1965) more recently reviewed the concept and significance of labile and over-all protein reserves. Protein reserves were defined as those tissue proteins which can be reversibly depleted and repleted so as to contribute to the free amino acid pools of the body. These authors stated that the labile protein reserves of the liver, intestines, pancreas and other viscera plus the less readily depleted tissue proteins are all part of the total reserves that can amount to as much as 25 percent of the body nitrogen.

Munro (1964) summarized studies on protein reserves and discounted the probability that labile nitrogen reserves

are in the form of free amino nitrogen. In order to explain total labile nitrogen, tissue levels of free amino nitrogen would have to approximate 100 mg/100 g body weight. Tissue levels of well-fed animals usually contain 20 to 40 mg/100 g. Furthermore, depletion of reserves does not markedly reduce tissue free amino acid levels. Munro (1964) concluded that protein reserves must be in the form of proteins. Three major factors stimulating deposition of labile protein are (1) increased dietary protein level, (2) protein-sparing action of dietary carbohydrate, and (3) action of anabolic hormones.

Poo et al. (1940) concluded that the extent of maternal protein storage in the pregnant rat was dependent on protein intake. At low intakes (1 g/day) pregnant rats had less storage (carcass) protein than non-pregnant controls, but at higher protein intakes (2 or 3 g/day) pregnant rat carcasses contained more protein than controls. At the end of pregnancy, the distribution of protein in the body was not appreciably influenced by protein intake. Approximately 8 percent of total body protein was found in the uterus and embryos.

Campbell et al. (1953) reduced food intake during the last 6 days of pregnancy in the rat and found no decrease in number or weight of fetuses or placentae. The deficit in energy and protein was compensated for by maternal tissues which lost weight during the reduced intake period. Maternal reserves which were accumulated during the first

13 to 15 days of pregnancy were apparently readily available for growth of the fetuses during the last week.

Naismith (1973) and Naismith and Morgan (1974) suggested increased food intake and improved efficiency of protein metabolism as the mechanisms by which the pregnant rat can store protein. During the last week of pregnancy, these protein stores are catabolized and used for fetal growth. Supplementation of protein to the dam in early pregnancy resulted in increased body weight, tissue protein, and tissue cellularity in the fetus. Improved nutrient transport through larger placentae may also have contributed to growth of the fetus.

Duncan and Lodge (1960) cite several studies in which the amount of nitrogen retained during the gestation of a sow greatly exceeds the amount accounted for by gains in the uterus and mammary region. After parturition much of the excess protein stored in the body may be lost independently of milk production. These authors concluded that it might be unwise to base requirements for nitrogen on the amount accumulated in uterine and mammary tissues. A further allowance for storage protein may be desirable for optimal performance.

Elsley et al. (1966) suggested that early pregnancy anabolism accounted for the increased liveweight gains of pregnant versus non-pregnant gilts. However, by the end of gestation, the difference in liveweight (25 kg) was more than accounted for by increases in blood, mammary tissues,

reproductive tract and its contents. If early pregnancy anabolism included protein storage, translocation to the site of fetal needs may have followed in late gestation.

The ham muscles of pregnant gilts have been found to contain less protein and more water than non-pregnant controls when slaughtered at day 112 of gestation (Kline, et al., 1972). The authors suggested that protein was mobilized from muscular tissues to develop fetal tissues.

Heap and Lodge (1967) conducted similar experiments with pregnant and non-pregnant sows and showed small increases in muscle and fat tissues of carcasses at 110 days of gestation. The muscles of pregnant sows tended to contain less nitrogen and more water although numbers of animals were inadequate to show significant differences. These authors discounted the existence of pregnancy anabolism in the sow.

The extent, nature and physiological importance of protein reserves in the gravid gilt have not been elucidated at this time. Extrapolations of such data from rats and other species to the gilt may or may not be valid.

Relationships Among Dietary Protein and Tissue Levels of Proteins, DNA and RNA

Tissue Proteins

Evaluation of dietary protein adequacy through the use of tissue levels of proteins has several limitations.

Nutritional stresses can change either the concentration of protein or the absolute amount of protein per tissue. Also, some organs can be affected more than others, some types of tissues more than others and some proteins more than others. Waterlow (1969) has written extensively on the problems encountered in determining protein deprivation due to concurrent changes in levels of fat, water and minerals. These same problems may be encountered in any attempt to relate protein intake and stage of gestation in gilts to the level of protein found in body tissues.

Work by Mendes and Waterlow (1958) indicated that low protein intake reduced rat muscle protein nitrogen when expressed on a concentration of wet tissue basis. Non-collagen nitrogen decreased while collagen nitrogen increased as a percentage of total nitrogen. Subsequent repletion showed that muscle proteins were regenerated more slowly than the more labile liver proteins.

Other studies of general undernourishment of the pig and chicken (Widdowson et al., 1960; Dickerson and McCance, 1960; Dickerson and Widdowson, 1960) indicate that sarcoplasmic and fibrillar proteins are most sensitive to nutritional manipulation. Collagen proteins are only slightly affected by nutritional stress and thus could actually increase in proportion to other muscle proteins during malnourishment.

The differences in changes of levels of sarcoplasmic, fibrillar, and stromal (collagen) proteins are most likely

related to their turnover rates (Wannemacher and Cooper, 1970). Half-lives are cited as 6.5 days for sarcoplasmic globulin, 10 days for sarcoplasmic albumin, 25 to 80 days for myosin and actin and greater than 100 days for collagen. Reasons for these differences in turnover rates are related to rate of protein degradation (Goldberg and Dice, 1974; Low and Goldberg, 1973). A decreased rate of protein synthesis as found in dietary protein inadequacy would first affect tissue levels of those proteins with higher turnover rates, namely sarcoplasmic proteins.

Long term undernourishment did not affect the ratio of fibrillar to sarcoplasmic proteins with pigs (Widdowson et al., 1960) and rats (Waterlow and Stephen, 1966) but other studies have shown a greater loss of sarcoplasmic proteins (Dickerson and McCance, 1960).

In the opposite situation where we are concerned with the nature of stored protein in tissues, there is less information available. Goldberg (1968) studied work-induced hypertrophy of rat muscles and found synthesis of sarcoplasmic and fibrillar proteins to be similar in proportion to that in non-growing controls. However, due to different rates of degradation, the effects of hypertrophy on tissue are not known. The nature of the majority of protein reserves is one of slow depletion. This infers slow turnover and would point to fibrillar proteins as the major site of protein reserves.

This idea is supported by the work of Dickerson and

Widdowson (1960) which showed relative concentrations of nitrogen from total protein, fibrillar protein and sarcoplasmic protein in the pig. From mid-gestation to adulthood sarcoplasmic protein nitrogen changes only slightly whereas fibrillar protein nitrogen increases greatly and explains most of the increase in total protein nitrogen. If reserve protein is being deposited, it is likely to be in the form of fibrillar protein.

Tissue DNA Levels

Other cellular compounds have been widely used to indicate tissue protein metabolism responses to such stimuli as protein intake and pregnancy. DNA content of a tissue has been used as an indicator of relative nuclei numbers. The non-dividing nucleus has been estimated to contain a fairly constant 7×10^{-12} g of DNA (Munro and Fleck, 1969). This constancy of DNA per diploid set of chromosomes has been questioned by some researchers who have reported metabolically active DNA (Stroun *et al.*, 1967).

Mature skeletal muscle cells may contain from 100 to 200 nuclei; however, a limit exists to the amount of cytoplasm that a nucleus can maintain (Stromer *et al.*, 1974). Therefore an increase in muscle cell size ultimately requires an increase in nuclei numbers. Martin *et al.*(1974) have reported increases in muscle DNA beyond 6 months of age in the gilt and Robinson (1969) noted increases in pig muscle DNA at 100 days of age. This implies that gilts of breeding

age may be able to increase muscle tissue mass by increasing nuclei numbers (DNA) as described by Stromer et al. (1974). The use of ratios of protein to DNA or muscle to DNA is of questionable value to evaluate growth because the addition of new nuclei could theoretically lower this ratio. Growing tissues could have a wide range of protein to DNA ratios.

Another problem associated with using DNA as a reference point for tissue analysis is the fact that organs and tissues contain mixtures of cell types with different volumes of cytoplasm per nucleus (Munro and Fleck, 1969). This problem may be minimal in muscle tissue but the pregnant uterus would contain a mixture of cell types, some of which would be actively dividing (more than diploid). Venable (1966) reported that of the nuclei found in the levator ani muscle of the mouse, only 57 percent belonged to contractile cells. Thus, the protein:DNA ratio may not be a valid index of protein metabolism with respect to sarcoplasmic and fibrillar protein.

Although various expressions of DNA may help us learn more about tissue growth, the use of total DNA per muscle (Trenkle, 1974) or organ (i.e. uterus, Leathem et al., 1968) appears to be the statistic least vulnerable to distortion by changes in other tissue constituents.

Tissue RNA Levels

The use of RNA levels in tissues as an indicator of protein synthetic activity is well documented. Bergen (1974)

summarized recent work on regulation of protein synthesis through control of RNA levels. Amino acid deficiencies (protein deprivation) reduce synthesis of ribosomal RNA, possibly through an accumulation of unacylated tRNA. Reduction of ribosomal RNA has the long-term effect of reducing protein synthesis. Allsion et al. (1963) and Howarth (1972) have shown reduced overall RNA levels in muscles of protein deficient rats. Leathem et al. (1968) found uterine RNA to be less responsive to pregnancy in the rat when poor quality proteins were fed.

Reduced RNA:DNA ratios also serve as good indicators of reduced protein synthetic activity in muscle. More specifically, ribosomal RNA concentration is a major determinant of muscle protein syntheis.

One complication encountered in using RNA levels to indicate protein synthetic activity is that of altered efficiencies of synthesis. The amount of cellular protein supported per unit of total RNA increases as growth progresses (Young, 1974). Bergen (1974) states that efficiency of protein synthesis (amino acids incorporated per unit of ribosomal RNA) is probably controlled by hormonal factors which may account for increased protein synthesis associated with endocrine changes of pregnancy.

CHAPTER III

GROWTH, REPRODUCTIVE PERFORMANCE AND NITROGEN BALANCE OF GILTS AS AFFECTED BY PROTEIN INTAKE AND STAGE OF GESTATION[1,2]

Summary

Two trials were conducted to establish the effects of four levels of crude protein and three stages of gestation of the growth, reproductive performance and nitrogen balance of 68 crossbred gilts. Gilts were fed 147, 256, 309, or 369 g of crude protein daily in trial 1 and 119, 227, 275 or 326 g per day in trial 2. Total body weight gain and average daily gain were not significantly affected by protein intake. Total gain and average daily gain increased linearly ($P<.01$) as stage of gestation progressed.

Gilts were slaughtered at 30, 60, or 90 days of gestation and evaluated for reproductive performance. Protein intake did not significantly affect number of corpora lutea,

---

[1]Journal Article of the Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater 74074.

[2]The authors wish to express appreciation to Rex Vencl for his care of experimental animals and R.K. Johnson for assistance with statistical analysis.

number of live embryos, survival rate or embryo lengths. Survival rate ($P<.05$), number of corpora lutea ($P<.05$) and number of live embryos ($P<.01$) declined linearly as gestation stage progressed. Embryo length adjusted for differences in embryo age appeared to increase at a decreasing rate (quadratic, $P<.01$) through gestation indicating that some factor limited growth in length of the fetus as early as 90 days of gestation.

Nitrogen balance studies were conducted prior to breeding and in early, mid and late gestation. Gestational balance data were affected by interactions possibly explained by seasonal temperature effects on energy requirements of the gilt. In trial 1 (conducted from March 1, 1974 to September 4, 1974), increasing dietary protein resulted in a linear ($P<.01$) increase in nitrogen retention. Retention increased ($P<.01$) by 4.1 g/day from pre-breeding to early pregnancy studies. Stage of gestation had a quadratic effect on retained nitrogen; retention was higher in early and late gestation than in mid gestation.

Trial 2 was conducted from January 1, 1975 to June 5, 1975 and overall mean nitrogen retention during pregnancy was -1.7 g/day. Retention was not significantly affected by protein intake but retained nitrogen declined in a quadratic ($P<.05$) manner over stages of gestation to a -16.3 g/day in late gestation. Catabolism of protein for energy of maintenance and increasing fetal energy needs in late gestation may explain these results. Unlike trial 1,

inception of pregnancy did not result in an increase in retention of nitrogen. No relationship between protein intake and nitrogen balance data and subsequent reproductive performance could be established. The lowest level of protein intake (147 g/day) appeared to be adequate for the first 90 days of gestation in the gilt. However, nitrogen retention was not maximized at this level of protein intake and subsequent lactation may have been adversely affected. Energy levels in diets fed in this experiment were apparently inadequate to support nitrogen retention in trial 2 when environmental temperatures were lower. N.R.C. (1973) recommendations of 6600 kcal/day may well be a lower limit for digestible energy requirements in gestating gilts during cooler seasons.

Introduction

In establishing recommended protein allowances for reproducing gilts, a review of the literature reveals no sound relationships between protein intake levels or protein source and subsequent litter size or pig birth weight (Clawson et al., 1963; Rippel et al., 1965a; Frobish et al., 1966; Baker et al., 1970; Hawton and Meade, 1971; Degeeter et al., 1972).

Work by Pond et al. (1968) suggests that protein limitation has its greatest effect on pregnancy during the first 3 or 4 weeks. Jones and Maxwell (1974) found a linear increase in number of corpora lutea as protein intake

increased from 143 to 345 g/day in gilts fed from before puberty; however, number of live embryos and survival rate at 30 days of gestation were not significantly affected by protein level. Severe protein restriction reduced reproductive efficiency of gilts as measured by percent exhibiting estrus, time interval to estrus and ovulation rate at the second pregnancy (Svajgr et al., 1972). Jones and Maxwell (1974) also noted a tendency toward higher anestrous rates in gilts fed lower protein levels.

Nitrogen balance of gilts during late gestation has been documented by Rippel et al. (1965b), Miller et al. (1969), and Hesby et al. (1971) with little evidence of a relationship between nitrogen retention and parturition performance. Miller et al. (1969) reported linear increases in nitrogen retained at 100 days of gestation as protein intake increased from 114 to 342 g/day. During early gestation, a similar linear increase has been noted by Jones and Maxwell (1974). The effect of stage of gestation on nitrogen balance has been reported as a linear increase throughout gestation (Elsley et al., 1966; Kline et al., 1972).

The objectives of this study were to examine nitrogen retention in the pregnant gilt as affected by protein intake and stage of gestation. Concurrent evaluation of reproductive performance and growth rate were conducted.

Materials and Methods

Two trials used a total of 68 crossbred (Hamp-York and Hamp-York-Duroc) gilts randomly allotted by weight to one of four protein treatment groups and subsequently to one of three slaughter groups (Table I). Gilts were approximately 275 days of age at the start of the trial. Treatment groups were assigned diets calculated to contain 8, 14, 17 and 20% crude protein. The 20% crude protein corn-soybean meal diet was extended with corn starch to give 17, 14, and 8% crude protein diets while retaining amino acid ratios across protein levels (Table II). Dietary calcium and phosphorus were maintained at .75 and .50% respectively, and vitamins and trace minerals were supplied at levels presented in Table II. Slaughter groups 30, 60 and 90 represent the approximate days after breeding when gilts were slaughtered.

From cursory observations, groups of gilts were observed to have been exhibiting estrus before the experiment began. Gilts were allotted to treatment groups and as each individual gilt exhibited estrus, the rations were changed from 2.27 kg of a 16% crude protein diet to 1.82 kg of the experimental diet. This provided daily crude protein and digestible energy intakes for the respective trials as shown in Table III. Trial 2 crude protein levels were approximately 88% of calculated values apparently due to corn or soybean meal crude protein values below expected levels. Each of the four treatment groups was randomly assigned to

one of four dirt lots. Gilts were fed once daily in individual feeding stalls and had access to drinking water and shelter. In summer, gilts were provided with foggers for cooling and in winter, a minimal amount of wheat straw bedding was used. Gilts were observed daily for signs of estrus by introducing a teaser boar into the pens. Each gilt was mated to two different boars on consecutive days of the second estrus on trial.

Five-day nitrogen balance studies were conducted on a random sample (47) of the gilts. Collection periods 1, 2, 3, and 4 were defined as those before breeding, early, mid, and late gestation, respectively. Animals were placed in metal metabolism stalls on day 11 after the first estrus on trial and again on day 21 after breeding. Gilts in slaughter groups 60 and 90 were placed in stalls on day 51 of gestation and gilts in slaughter group 90 were returned to stalls on day 81 of gestation with a 2-day adjustment period prior to each 5-day collection. Urine was collected in concentrated hydrochloric acid and 10% of daily urine and fecal collections were frozen for Kjeldahl nitrogen analysis (A.O.A.C., 1960).

Gilts were weighed at the start of the trial and at slaughter. Gilts were salughtered at least once weekly, as near allotted slaughter dates as possible. Mean embryo ages are given in Table VI. Reproductive tracts were recovered and evaluated immediately. Corpora lutea counts were recorded for each ovary and each uterine horn was cut open,

starting with the cervical end, to obtain embryos. Crown to rump length was measured with calipers on all viable 30-day embryos while encased in the amnionic sac. Sixty and ninety-day embryos were separated from placental membranes and allowed to assume a "natural" position before measurement of crown to rump lengths. Viable embryos were considered to be those which showed no signs of atrophy and were of reasonably normal size.

Data were analyzed by least squares analysis of variance described by Harvey (1960). Nitrogen balance data were analyzed as split-plots (Table IV) within each trial due to significant trial by protein level by collection period interactions. To compare nitrogen balance in open and early pregnancy gilts, an analysis was conducted with a model including four protein levels and two collection periods. An F-test of the collection period sums of squares provided probability levels for differences. Growth and reproductive performance data were analyzed as a randomized block with a model including two trials, four protein levels and three slaughter groups (stages of gestation). Trial interactions were not indicated in growth and reproductive performance data; therefore, the trial results were pooled for analysis. Embryo numbers and survival rates were adjusted for unequal number of corpora lutea and embryo length was adjusted for unequal age of embryos by partial regression procedures of the least squares program (Harvey, 1960). All least squares means were tested for fit to

linear, quadratic and cubic polynomials over the four protein levels and linear or quadratic for the three collection periods or slaughter groups.

Results and Discussion

Growth

Growth data for the combined trials are presented in Table V. Protein level did not significantly affect mean weight gain from the start of the trial to slaughter date which averaged 111.6 days for all gilts. Mean weight gains were 20.1, 22.4, 26.4, and 22.4 kg for protein groups 8, 14, 17, and 20, respectively. Protein level tended to have a quadratic effect on average daily gain ($P<.10$). Rates of gain in this experiment were considerably lower than that observed by Baker et al. (1969) in gestating gilts fed 1.9 kg of a 16% crude protein diet. However, Baker et al. (1969) measured gain over the entire gestation period and cited the fact that pregnant gilts have a greater propensity for weight gain than nonpregnant gilts at similar intakes.

Growth during gestation was linear ($P<.01$) as measured by slaughter weight and total gain. The average daily gain for the three slaughter groups more clearly describes growth and indicates that as gestation progressed, rate of gain increased linearly ($P<.01$). The longer a gilt was in the state of pregnancy, the greater the overall rate of gain due to lesser dilution by prepregnancy rate of gain. Elsley

et al. (1966) presents a growth curve with similar shape but greater magnitude for bred gilts fed 2.2 kg/day of a 14% crude protein diet. Although gilts in trial 1 initially weighed 22.6 kg more ($P<.01$) than trial 2 gilts, weight gain patterns were similar.

Reproductive Performance

Table VI summarizes the reproductive performance for combined trials classified by protein level and slaughter group. None of the measurements obtained were affected by protein intake. Numbers of corpora lutea were 12.6, 13.4, 13.0, and 13.8 for protein levels 8, 14, 17, and 20%, respectively. Mean ovulation rate (as indicated by corpora lutea) was 13.2 for all gilts, slightly lower than the 13.8 corpora lutea reported previously by Jones and Maxwell (1974). Number of corpora lutea appeared to decrease with stage of gestation (linear, $P<.05$); however, since there were no repeated observations of corpora lutea in the same animals, it may be wise to disregard this decline in luteal count with progressing pregnancy. There was a decline in number of embryos (2.9 embryos) and corresponding decline in corpora lutea (1.4 corpora lutea) as pregnancy progressed from 30 to 90 days. Polge et al. (1966) observed the effects of reducing numbers of embryos during early gestation on the maintenance of pregnancy and suggested that four or more embryos were necessary to overcome the uterine luteolytic effect. It is possible that as embryo numbers declined due to normal mortality within the uterus, a small degree of

luteolytic activity caused regression of the smaller, underdeveloped corpora lutea. These data are not adequate to substantiate such an occurence.

Numbers of live embryos were not affected by protein intake with 11.1, 11.0, 10.7, and 10.2 embryos for 8, 14, 17, and 20% diets, respectively. Partial regression of live embryo numbers on numbers of corpora lutea resulted in a significant reduction of the error mean square indicating a part of the variation in live embryos was associated with differences in corpora lutea numbers. However, protein levels had no effect on number of live embryos at 30, 60, or 90 days postbreeding. This is in agreement with earlier work by Jones and Maxwell (1974) at 30 days postbreeding. As gestation progressed, number of live embryos declined in a linear ($P<.01$) manner. Number of embryos adjusted for unequal numbers of corpora lutea also declined linearly ($P<.05$).

Survival rate or percentage of corpora lutea represented by live embryos at slaughter was not significantly affected by protein intake, although survival rate declined by 13.7% as protein level was increased from 8 to 20%. As expected, survival rate declined at each successive stage of gestation (linear, $P<.05$). Mean embryo survival at 30 days in this experiment (88.6%) was higher than the 57 to 70% at 25 days cited in a review by Hanly (1961).

After adjusting for differences in embryo length which were associated with age of embryos, protein level was found

to have no significant effect on length. Since the observed quadratic effect ($P<.05$) of stage of gestation on embryo length was independent of age, some other factor, such as number of embryos in the litter, must have been involved. These data suggest that factors limiting growth were present at day 90 because the rate of increase in length was declining. However, it is possible that the very nature of growth of the fetus at later stages of gestation does not lend itself to evaluation by crown-rump length measurements.

Nitrogen Balance

Significant trial by protein level by collection period interactions warranted discussion of nitrogen balance studies on a within trial basis. Least squares means for nitrogen retained, urinary nitrogen, fecal nitrogen, and retention efficiency are given in Table VII. Retention efficiency or biological value is defined as retained nitrogen expressed as a percentage of absorbed nitrogen and should indicate the degree of efficiency with which absorbed nitrogen is used for tissue gains. Since collection period 1 (before breeding) involves the gilt in an entirely different physiological state from other collection periods, a separate analysis of the data was conducted to compare nitrogen balance of the open gilts (collection period 1) versus gilts in early pregnancy (collection period 2). These data are presented in Table VIII.

Trial 1 was begun on March 1, 1974 and continued until

September 4, 1974 and all 24 gilts were subjected to nitrogen balance studies. As protein level increased, nitrogen retention increased linearly ($P<.01$) with mean values of 10.3, 18.1, 22.1, and 28.9 g/day for protein levels 8, 14, 17, and 20%, respectively. This linear relationship is similar to that reported by Miller et al. (1969) during late gestation and Jones and Maxwell (1974) in early gestation. Values reported here are least squares means of retention at three stages of gestation and comparable values are not found in the literature. Retention of nitrogen appeared to be reduced at mid-gestation (collection period 3) but similar in early and late gestation as evidenced by a significant quadratic, but not linear, relationship. It is possible that nitrogen storage initiated by pregnancy brought reserves to near capacity so as to somewhat reduce retention in mid-gestation, followed by increased retention in late pregnancy to meet increased fetal demands. These data conflict with earlier work by Elsley et al. (1966) and Kline et al. (1972) which showed increasing retention from a low value early in gestation to maximum retentions in late gestation. In this experiment, all diets had amino acid ratios similar to a high quality 20% crude protein mixture, possibly accounting for improved retention by providing substrate stimulation of rRNA and protein synthesis as described by Bergen (1974).

Urinary nitrogen reflected retained nitrogen with a linear ($P<.01$) increase as protein intake increased and a

similar, but inverted, quadratic ($P<.01$) response to stage of gestation. Fecal nitrogen responded in a linear ($P<.05$) manner to protein intake; however, a quadratic ($P<.10$) trend was apparent due to lower values for protein levels 8 and 20%. Retention efficiency did not appear to be affected by protein intake and had a mean value for all gilts of 49.8%. Retained nitrogen was reflected by a similar quadratic ($P<.05$) response of retention efficiency to stage of gestation. Changes in retention efficiency suggest changes in protein synthesis. Bergen (1974) states that total protein synthesis can only be increased by increasing the synthetic capacity (elevated rRNA) through improved substrate availability and hormonal influence or by increasing the efficiency of synthetic machinery through hormonal factors. If stage of gestation affects protein synthesis, the control mechanism would probably be through hormonal modification of RNA synthesis (Manchester, 1970) or through an increased rate of ribosome movement along mRNA (Manchester, 1972). The exact control is not yet understood.

In trial 1, a comparison of nitrogen balance of pregnant versus nonpregnant gilts demonstrated significantly increased retention and retention efficiency for pregnant gilts (Table VIII). Conception increased nitrogen balance by 4.1 g/day. Urinary nitrogen was significantly decreased by pregnancy while fecal nitrogen was not affected, indicating that improved absorption of nitrogen was not involved in the increased retention efficiency. Elsley

et al. (1966) reported a mean increase in retained nitrogen of 4.6 g/day for pregnant versus nonpregnant gilts in six 10-day balance studies spaced throughout pregnancy.

Trial 2 was conducted during the winter and spring, starting on January 1, 1975 and ending June 5, 1975. Twenty-three of the forty gilts were subjected to nitrogen balance studies. Nitrogen balance in this trial was considerably lower than in the previous trial with overall mean retention of -1.7 g/day versus 19.8 g/day for trial 1. Retention was not significantly affected by protein intake, but there was a significant quadratic effect of stage of gestation on retention. Mean retentions of 7.3, 4.1, and -16.3 g/day were recorded in collection periods 2, 3, and 4, respectively. Possibly the cooler temperatures of this season increased the gilts' maintenance energy requirements to such a degree that protein was being catabolized as an energy source with subsequent loss of nitrogen. The increasing needs of the developing fetuses could have placed further demands for energy on the dam as demonstrated by the high negative nitrogen balance in collection period 4. Calculated daily intakes of digestible energy were 6323, 6255, 6223, and 6190 kcal/day for protein levels 8, 14, 17, and 20%, respectively. Although these values are quite similar, they are below the N.R.C. (1973) recommendation of 6600 kcal/day for gilts. Munro (1964) reviewed the influence of dietary energy on nitrogen balance and summarized the available data by stating that under normal nutritional

conditions, nitrogen retention is increased by an increase in energy intake as long as protein intake is adequate. Conversely, an increase in protein intake may not result in increased nitrogen retention if energy intake is inadequate. Studies with adult rats, dogs, and humans showed that within limits, additional energy resulted in linear increases in nitrogen retention (2 to 4 mgN/kcal). Therefore, it is possible that energy intake in trial 2 was inadequate to allow maximal retention of nitrogen. Baker et al. (1969) found that at least 1.9 kg/day of a 16% crude protein corn soybean meal diet was necessary to maximize pig birth weights indicating that this level of intake provides adequate combinations of energy and protein to maximize intrauterine growth. Levels of intake in this experiment (1.82 kg/day) would appear slightly marginal in light of their work. Trial 2 crude protein intakes were lower than trial 1 values due to a lower than expected protein content of the corn or soybean meal.

Urinary and fecal nitrogen increased linearly ($P<.01$) with increasing protein intake and urinary nitrogen increased in a quadratic ($P<.01$) fashion over stages of gestation. Fecal nitrogen responded to stage of gestation in a quadratic ($P<.05$) manner with apparent digestion being greatest in late gestation. Retention efficiency was not significantly affected by protein intake, but there was a quadratic ($P<.05$) response to stage of gestation. Retention efficiency, or the percentage of absorbed nitrogen

that is retained, would theoretically decrease when the amount absorbed exceeds the amount which can be used for protein synthesis in the body, or if amino acids are being used for gluconeogenesis.

A comparison of gilts before and after breeding did not show an increased nitrogen retention with pregnancy as seen in trial 1. Mean retentions were 8.6 and 6.8 g/day for collection periods 1 and 2, respectively.

TABLE I

NUMBERS OF GILTS BY TRIAL, PROTEIN LEVEL AND SLAUGHTER GROUP

Slaughter Group[a]	Protein Level 8%	14%	17%	20%	Total
		Trial 1			
30 Days	3	2	2	3	10
60 Days	2	2	2	2	8
90 Days	1	2	2	1	6
Total	6[b]	6[c]	6[c]	6[d]	24
		Trial 2			
30 Days	2	3	3	3	11
60 Days	3	3	3	2	11
90 Days	4	4	4	3	15
Total	9[c]	10	10	8[c,d]	37

[a]Slaughter group number indicates approximate date of gestation when slaughtered.

[b]One gilt apparently pregnant before trial began.

[c]One gilt anestrous.

[d]One gilt aborted due to injury.

TABLE II

CALCULATED COMPOSITION OF EXPERIMENTAL DIETS

Ingredients	International Reference No.	8% Protein	14% Protein	17% Protein	20% Protein
Corn (9%)[a] Corn, dent yellow, grain, gr2US mn54wt, (4)	4-02-931	24.75	44.76	54.77	64.77
Soybean meal (44%) Soybean, seed, solv-extd grnd, mx 7fbr (5)	5-04-604	12.31	22.25	27.23	32.20
Corn starch (0.6%) Corn, starch, dehy grnd, (4)	4-02-889	59.24	29.62	14.80	---
Dicalcium phosphate Calcium phosphate, dibasic, commercial	6-01-080	1.94	1.33	1.02	.71
Calcium carbonate $CaCO_3$, commercial mn 38% calcium	6-01-069	.76	1.04	1.18	1.32
Vitamin T.M. Premix		.50	.50	.50	.50
Salt		.50	.50	.50	.50

[a]Estimated percent protein given in parentheses.

[b]Vitamin-trace mineral premix supplied 3300 USP Units Vitamin A, 33 USP Units Vitamin D_3, 6.6 IU Vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 33 mg niacin, 1100 mg choline chloride, .0165 mg Vitamin B_{12}, 2.2 mg menadione sodium bisulfite per kg of diet and 22 PPM Mn, 100 PPM Zn, 122 PPM I, 100 PPM Fe and 11 PPM Cu.

TABLE III

DAILY NITROGEN, CRUDE PROTEIN AND DIGESTIBLE ENERGY INTAKES

Item	Protein Level			
	8%	14%	17%	20%
Calculated N[a]	23.3	40.8	49.5	58.2
Actual N[a]	23.5 (10.5)	40.9 (36.3)	49.5 (44.0)	59.0 (52.1)
Calculated Crude Protein[a]	146	255	309	364
Actual Crude Protein[a]	147 (119)	256 (227)	309 (275)	369 (326)
Calculated Digestible Energy (kcal/day)	6323	6255	6223	6190

[a]Values given in g/day with trial 2 values in parentheses.

TABLE IV

ANALYSIS OF VARIANCE TABLE FOR
NITROGEN BALANCE DATA

Source	Degrees of Freedom	
	Trial 1	Trial 2
Total (Corrected)	43	51
Between animals	23	22
Protein levels	3	3
Linear	1	1
Quadratic	1	1
Cubic	1	1
Animals/protein levels	20	19
Within animals	20	29
Collection periods	2	2
Linear	1	1
Quadratic	1	1
Protein level x Collection period	6	6
Animals x Collection period/ protein level	12	21

TABLE V

BODY WEIGHT AND GROWTH DATA OF GILTS[a]

Item	Protein Level				Slaughter Group		
	8%	14%	17%	20%	30	60	90
No. of Gilts	15	16	16	14	21	19	21
Initial Weight (kg)	133±2.9	128±2.8	130±2.8	130±3.0	131±2.4	131±2.5	128±2.7
Slaughter Weight (kg)	153±2.9	150±2.8	156±2.8	152±3.0	145±2.4	154±2.5	160±2.7[b]
Total Gain (kg)	20.1±2.5	22.4±2.4	26.4±2.4	22.9±2.6	13.2±2.1	23.7±2.2	32.0±2.3[b]
Average Daily Gain (kg)	.173±.022	.221±.021	.225±.021	.191±.023	.156±.018	.205±.019	.264±.020[b]

[a]Means ± standard error.

[b]Linear effect significant ($P<.01$).

[c]Quadratic effect approached significance ($P<.10$).

TABLE VI

REPRODUCTIVE PERFORMANCE OF GILTS[a]

Item	Protein Level				Slaughter Group		
	8%	14%	17%	20%	30	60	90
No. of gilts	15	16	16	14	21	19	21
No. corpora lutea	12.6±.56	13.4±.54	13.0±.54	13.8±.58	14.0±.46	13.0±.48	12.6±.51[b]
No. live embryos	11.1±.66	11.0±.64	10.7±.64	10.2±.69	12.4±.54	10.3±.57	9.5±.61[c]
No. embryos adjusted[e]	11.4±.61	10.9±.58	10.8±.58	9.8±.63	11.9±.51	10.4±.52	9.8±.56[b]
Actual survival rate (%)	86.9±4.7	82.5±4.5	82.7±4.5	73.2±4.8	88.6±3.8	80.0±4.1	75.4±4.3[b]
Adjusted survival rate (%)[e]	85.9±4.7	82.9±4.5	82.4±4.5	74.3±4.9	90.1±4.0	79.7±4.0	74.4±4.3[b]
Embryo length (mm)[f]	136.8±1.80	139.1±1.79	137.0±1.75	141.6±1.85	109.2±13.7	144.3±1.6	163.5±13.1[d]
Embryo ages (days)	62.4±.6	62.1±.6	62.0±.6	62.9±.6	32.2±.5	62.9±.5	92.0±.6

[a]Means ± standard errors.
[b]Linear effect significant ($P < .05$).
[c]Linear effect significant ($P < .01$).
[d]Quadratic effect significant ($P < .01$).
[e]Values adjusted to overall corpora lutea mean of 13.2.
[f]Values adjusted to overall embryo age of 62.8 days.

TABLE VII

NITROGEN BALANCE OF GILTS FED GRADED LEVELS OF PROTEIN[a]

Item	Protein Level				Collection Period		
	8%	14%	17%	20%	2	3	4
			Trial 1				
No. of gilts	6	6	6	6	24	14	6
Retained N (g/day)	10.3±2.0	18.1±1.7	22.1±1.7	28.9±2.0[b]	19.9±1.1	17.8±1.2	21.7±1.6[d]
Urinary N (g/day)	11.0±2.1	18.2±1.7	22.8±1.7	25.7±2.1[b]	19.1±1.1	21.9±1.2	17.3±1.6[e]
Fecal N (g/day)	2.2±.7	4.6±.6	4.7±.6	4.4±.7[c]	4.2±.4	3.5±.4	4.2±.6
Retention efficiency (%)	47.2±5.4	50.6±4.4	48.9±4.4	52.6±5.4	49.9±2.8	44.1±3.0	55.4±4.1[d]
			Trial 2				
No. of gilts	5	6	6	6	23	18	11
Retained N (g/day)	.8±3.8	4.0±3.6	-8.0±3.6	-3.6±4.0	7.3±2.8	4.1±3.0	-16.3±3.9[d]
Urinary N (g/day)	15.1±3.3	26.9±3.1	4.15±3.1	49.3±3.5[b]	23.4±2.3	26.5±2.5	49.7±3.2[e]
Fecal N (g/day)	3.1±.6	5.3±.6	5.6±.6	6.4±.6[b]	5.4±.3	5.5±.3	4.4±.4[d]
Retention efficiency (%)	6.8±10.5	13.0±9.9	-8.4±9.9	-7.6±11.2	27.7±7.5	18.2±8.0	-43.0±10.4[d]

[a]Means ± standard error.

[b]Linear effect significant (P<.01).

[c]Linear effect significant (P<.05).

[d]Quadratic effect significant (P<.05).

[e]Quadratic effect significant (P<.01).

TABLE VIII

NITROGEN BALANCE OF OPEN AND EARLY PREGNANCY GILTS[a]

Item	Protein Level				Collection Period	
	8%	14%	17%	20%	1[d]	2[e]
			Trial 1			
No. of gilts	6	6	6	6	24	24
Retained N (g/day)	7.5±1.4	15.2±1.4	21.9±1.4	27.3±1.4[b]	15.9±1.0	20.0±1.0[c]
Urinary N (g/day)	13.1±2.0	20.5±2.0	22.0±2.0	29.1±2.0[b]	23.5±1.3	18.9±1.3[c]
Fecal N (g/day)	2.9±.5	5.3±.5	5.6±.5	5.3±.5[b]	5.1±.3	4.4±.3
Retention efficiency (%)	36.2±3.9	42.2±3.9	49.7±3.9	51.0±3.9[b]	39.6±2.7	50.0±2.7[c]
			Trial 2			
No. of gilts	5	6	6	6	23	23
Retained N (g/day)	2.2±2.9	9.0±2.6	6.3±2.6	13.2±2.6	8.6±1.7	6.8±1.7
Urinary N (g/day)	13.3±2.0	21.3±1.8	27.9±1.8	31.3±1.8[b]	23.0±1.3	23.9±1.3
Fecal N (g/day)	3.4±.5	5.9±.4	6.4±.4	7.4±.4[b]	6.1±.3	5.5±.3
Retention efficiency (%)	14.0±6.8	29.9±6.2	25.4±6.2	34.3±6.2	26.0±4.1	25.9±4.1

[a]Means ± standard errors.
[b]Linear effect significant. (P<.01).
[c]Collection period differences significant (P<.01).
[d]Open collection period.
[e]Early pregnancy collection period.

CHAPTER IV

TISSUE LEVELS OF PROTEINS, DNA AND RNA AS AFFECTED BY PROTEIN INTAKE AND STAGE OF GESTATION IN GILTS[1,2]

Summary

Forty crossbred gilts were fed diets providing 119, 227, 275, or 326 g of crude protein from the estrus prior to breeding until slaughter at 30, 60, or 90 days of gestation. At slaughter, reproductive tracts, muscles of the ham (semitendinosus and biceps femoris) and the liver were removed, weighed and a sample of each were frozen for later analyses. Gravid uterine tissues and muscle samples were analyzed for sarcoplasmic and fibrillar proteins, crude protein, DNA and RNA. Uterine fluid volumes were recorded and fluid and liver samples were analyzed for crude protein.

Muscle weights and total sarcoplasmic protein increased linearly ($P<.05$) as stage of gestation progressed, while total muscle fibrillar protein and total crude protein

---

[1]Journal Article of the Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater 74074.

[2]The authors wish to express appreciation to Rex Vencl for his care of experimental animals and R.K. Johnson for assistance with statistical analysis.

increased in a linear ($P<.05$) manner as daily protein intake was increased.

A linear ($P<.01$) increase in gravid uterine tissue weight was apparent as stage of gestation progressed and was reflected by similar increases in fibrillar, sarcoplasmic, and crude protein values. Percentages of fibrillar and sarcoplasmic proteins increased (linear, $P<.05$) as protein intake increased.

Liver crude protein values did not support any pattern of protein storage or depletion during the first 90 days of gestation. Total uterine fluid crude protein increased greatly by mid-gestation and decreased slightly by 90 days of pregnancy (quadratic, $P<.01$).

Muscle RNA to DNA ratios increased (linear, $P<.05$) as pregnancy progressed as did total RNA ($P<.01$) suggesting increased protein synthetic activity. The lack of response of DNA to protein intake and stage of gestation indicates that increases in muscle weights and fibrillar and sarcoplasmic proteins were not due to increase in nuclei numbers but were simply hypertrophy of existing nucleus-cytoplasm units.

Total DNA and RNA of the gravid uterus increased (linear, $P<.01$) as pregnancy continued and the ratio of RNA and DNA was not significantly changed from 30 to 90 days of pregnancy, possibly reflecting hyperplasic fetal growth during this period.

Introduction

Tissue composition has been widely used to indicate dietary needs for growth. Waterlow (1969) reviewed the problems faced in evaluating dietary protein adequacy through the use of tissue protein levels. Mendes and Waterlow (1958) indicated that low dietary protein intake by rats reduced muscle protein nitrogen on a wet tissue basis. Sarcoplasmic and fibrillar proteins have been proposed as sensitive indicators of dietary protein adequacy (Widdowson et al., 1960; Dickerson and McCance, 1960; Dickerson and Widdowson, 1960) while collagen proteins are only slightly affected by nutritional stress. Relative turnover rates of different muscle proteins determine which will respond first to nutritional manipulation. Wannemacher and Cooper (1970) indicate that sarcoplasmic proteins have the greatest turnover rates followed by fibrillar proteins with collagen proteins having a much longer half-life. Low and Goldberg (1973) and Goldberg and Dice (1974) discussed reasons for differences in protein turnover rates with regard to rates of protein degradation. These authors concluded that protein structure determines degradation rates. In general, catabolism is more rapid for abnormal proteins, for larger molecular size proteins, for proteins altered by ligand binding, and for those proteins sensitive to certain proteases. Swick and Song (1974) more specifically reviewed the turnover rates of muscle proteins and

pointed out that only a small fraction of the total protein synthesized is accumulated during growth. These authors emphasized the importance of muscle protein turnover in enabling the animal to quickly adapt to a changing environment by catabolizing body tissues during times of inadequate nutrition.

Use of tissue protein levels for evaluating the adequacy of protein in gilt gestation diets is complex. Duncan and Lodge (1960), Elsley et al. (1966) and Kline et al. (1972) give evidence for an early pregnancy protein anabolism above that explained by uterine and mammary growth of the gilt. A similar protein storage has been demonstrated in the pregnant rat, whereby increased food intake and improved efficiency of protein metabolism have been implicated as mechanisms by which protein is stored during early pregnancy for use by the developing fetuses during the last week of pregnancy (Naismith, 1973; Naismith and Morgan, 1974). The extent, nature, and physiological importance of such protein reserves in the gravid gilt have not been elucidated.

DNA content of an organ or tissue is an indicator of relative nuclei numbers. The non-dividing nucleus has been estimated to contain a reasonably constant 7×10^{-12} g of DNA per diploid set of chromosomes (Munro and Fleck, 1969). Mature skeletal muscle cells may contain from 100 to 200 nuclei with a limited amount of cytoplasm maintained by each nucleus (Stromer et al., 1974). After increases in muscle cell numbers have ceased, additional nuclei may be added to

a cell to allow further growth of cell mass. Such an occurence would be indicated by an increase in total DNA per organ or tissue while concentration of DNA might remain unchanged. Martin et al. (1974) have reported increases in muscle DNA beyond 6 months of age in the gilt and Robinson (1969) noted increases in total pig muscle DNA at 100 days of age. This implies that gilts of breeding age may be capable of increasing muscle tissue mass by increasing nuclei numbers as described by Stromer et al. (1974).

DNA content can be used to indicate the relative growth of muscles during protein storage or growth of the uterus and its contents during gestation. However, interpretation of DNA data must recognize the fact that both muscles and the gravid uterus contain mixtures of cell types with different volumes of cytoplasm per nucleus and the uterus contains many actively dividing cells (more than diploid). Although various expressions of DNA may provide useful information about tissue growth, the use of total DNA per muscle (Trenkle, 1974) or organ (i.e. uterus; Leathem et al. 1968) appears to be the statistic least vulnerable to distortion by changes in other tissue constituents.

The use of tissue RNA levels as an indicator of growth and protein synthetic activity is well documented. Bergen (1974) summarized recent work on protein synthesis regulation through control of RNA levels. Evidence that RNA levels reflect dietary protein adequacy comes from work in which amino acid deficiencies have been shown to reduce

synthesis of ribosomal RNA, possibly through an accumulation of unacylated tRNA. Reduction of ribosomal RNA has a long term effect in reducing protein synthesis. Allison et al. (1963) and Howarth (1972) have shown reduced total RNA levels in muscles of protein deficient rats. Leathem et al. (1968) found uterine RNA levels to be less responsive to pregnancy in the rat when poor quality proteins were fed. RNA to DNA ratio serves as a good indicator of protein synthetic activity per unit of cytoplasm maintained by a nucleus. One problem associated with using RNA as a growth-protein synthesis indicator is the apparent difference in efficiency of protein synthesis (amino acids incorporated per unit of ribosomal RNA) possibly influenced by hormonal factors of pregnancy (Young, 1974; Bergen, 1974).

This experiment was conducted to supplement data from a nitrogen balance trial involving gilts fed graded levels of protein and slaughtered at three stages of gestation. The objectives of this experiment were to examine changes in protein, DNA and RNA levels in muscle tissue and gravid uterine tissue of gilts during gestation when fed four levels of protein.

Materials and Methods

Forty crossbred gilts (Hamp-York and Hamp-York-Duroc) approximately 275 days of age were randomly allotted to one of four protein treatment groups and subsequently to one of three slaughter groups (Table I). Treatment groups were

assigned diets calculated to contain 8, 14, 17, and 20% crude protein. The 20% crude protein corn-soybean meal diet was extended with corn starch to give 17, 14, and 8% crude protein diets with constant amino acid ratios across treatments (Table II). Dietary calcium and phosphorus were maintained at .75 and .50%, respectively; and vitamins and trace minerals were supplied at levels presented in Table II. Stage of gestation groups 30, 60, and 90 represent the approximate date of gestation when gilts were sacrificed.

Gilts were fed the allotted experimental diets from the estrus prior to breeding until slaughter. When fed at 1.82 kg/day, diets provided daily crude protein intakes of 119, 227, 275, and 326 g and digestible energy intakes of 6323, 6255, 6223, and 6190 kcal/day for protein treatment groups 8, 14, 17, and 20%, respectively. Crude protein intakes were approximately 88% of calculated values, apparently due to corn or soybean mean crude protein values lower than anticipated. Each of the four treatment groups was randomly assigned to one of four dirt lots and gilts were fed once daily in individual feeding stalls. Gilts were observed daily for signs of estrus by introducing a teaser boar into the pens. Each gilt was mated to two different boars on consecutive days at the second estrus on trial.

Nitrogen balance studies were conducted on a ramdom sample of the gilts (23 animals) as reported in Chapter III. Collections were conducted before breeding, and in early, mid, and late gestation, depending on date of slaughter.

Gilts were weighed at the start of the trial and at slaughter. Growth data is presented in Chapter III. Gilts were slaughtered as near allotted slaughter dates as possible. Reproductive tracts were recovered as quickly as possible after slaughter and uniformly trimmed by severing the uterus approximately 7.5 cm posterior to the junction of the uterine horns. The tracts were weighed and dissected for evaluation of reproductive performance (Chapter III). All solid tissues including the uterus, ovaries, placenta, and fetuses were ground twice through a 4.23 mm screen in a Hobart Model 4332 grinder and 100 g ground sample was quickly frozen in a dry ice-ethanol bath. Volumes were recorded for freely draining uterine fluids and a 10 ml sample was frozen for Kjeldahl nitrogen analysis. (A.O.A.C., 1960) The semitendinosus and biceps femoris muscles of each left ham were dissected as soon after slaughter as possible. The combined muscles were weighed, quickly ground, and 100 g samples frozen in a dry ice-ethanol bath. Liver weights were recorded and 20 g samples were frozen for Kjeldahl nitrogen analysis. (A.O.A.C., 1960)

Uterine and muscle tissues were analyzed for crude protein, sarcoplasmic protein, fibrillar protein, RNA and DNA. Duplicate 1 g samples of each tissue were subjected to Kjeldahl nitrogen analysis (A.O.A.C., 1960) and nitrogen values were multiplied by 6.25 to estimate crude protein content.

Sarcoplasmic and fibrillar proteins were determined by

a procedure similar to Helander (1957). Chips of the frozen uterine and muscle tissues weighing approximately 1 g were immersed in 10 ml cold .03 M KI + .03 M PO_4 buffer, pH 7.4. Tissues were extracted twice with this buffer in a Sorvall Omnimixer with a constant rheostat setting for 15 minutes. This rheostat setting was determined to be the maximum speed possible without excessive foaming. After each extraction, samples were centrifuged for 15 minutes at 3020 x G at 4 C in a Sorvall RCB 2 centrifuge. After centrifugation, the supernatant was filtered through glass wool. Proteins in combined filtrates were precipitated by addition of an equal volume of 20% trichloroacetic acid (TCA) and centrifugation at 4080 x G for 15 minutes. The precipitate (sarcoplasmic proteins) was frozen for Kjeldahl nitrogen analysis. (A.O.A.C., 1960) The residue, left after extraction by the first buffer, was then extracted twice with 10 ml volumes of 1.1 M KI + .1 M PO_4 buffer, pH 7.4. Extraction, centrifugation, and precipitation were carried out as before with the final precipitate being fibrillar proteins.

Uterine and muscle tissues were analyzed for RNA and DNA by procedures similar to those described by Tucker and Reece (1962). Approximately 5 g of ground tissue were weighed into a 50 ml plastic centrifuge tube and extracted for 24 hours with ethanol: chloroform (2:1 v/v) then with ethyl ether for 24 hours to remove lipids. Extraction was carried out on a shaker at room temperature. Tubes were

then centrifuged at 2000 x G for 15 minutes and the supernatant was discarded. The final residue was dried at 37 C for 6 hours then weighed and stored at -20 C. Samples were later ground through a 40 mesh screen in a Wiley Mill and again stored at -20 C.

Duplicate 25 mg samples were extracted with 5 ml of ethanol: chloroform (3:1 v/v) then extracted twice with 5 ml of ice-cold 10% aqueous TCA. TCA was removed from the residue by washing with 5 ml ice-cold ethanol saturated with sodium acetate. All extractions and washings were followed by centrifugation at 35,000 x G and 4 C in a Sorvall RCB 2 centrifuge. The final residue was digested in 3 ml of 1 N KOH for 15 hours in a 37 C waterbath-shaker. The resulting residue was acidified with .4 ml ice-cold 6 N HCL and 3.4 ml ice-cold 10% perchloric acid (PCA). The precipitate was centrifuged as before with the supernatant decanted and the resulting precipitate washed twice with 5 ml of 5% PCA. After centrifugation, the three supernatants were combined and volumes were standardized to 17 ml with 5% PCA. These combined supernatants were analyzed for RNA nucleotide bases by ultra violet absorption (260 nm) with a Gilford 240 spectrophotometer.

The final precipitate from RNA extraction was extracted twice for DNA with 5 ml of 5% PCA in a 70 C sand bath for 15 minutes. The combined supernatants were standardized to a 10 ml volume and analyzed for DNA nucleotide bases by ultra violet absorption (268 nm). Standard curves of

purified torula yeast RNA and highly polymerized calf thymus DNA (Sigma Chemical Company) were plotted and two standards were analyzed with each separate group of unknowns resulting in separate regression equations for calculation of unknown concentrations.

Data were analyzed by procedures described by Harvey (1960) due to unequal subclass size. Least squares means were tested for fit of linear, quadratic, and cubic polynomials over the four protein treatment levels and linear and quadratic fit over the three stages of gestation. Table IX shows a typical analysis of variance table.

Results and Discussion

Muscle Proteins

Table X indicates that protein intake did not influence wet weights of combined semitendinosus and biceps femoris muscles, total g of sarcoplasmic proteins, and wet tissue basis percentages of fibrillar, sarcoplasmic and crude protein. However, total fibrillar protein per muscle increased linearly ($P<.05$) as protein intake increased. This effect is apparently due to a combination of slight increases in muscle weight and fibrillar protein percentages which were not detectable with the numbers of animals involved in this experiment. A difference of 22.7 g of fibrillar protein between the 8 and 20% groups appears insignificant when compared to 192 g difference in muscle

weights observed and does not substantially support the concept of protein storage. It was anticipated that if protein storage had occurred, such storage would have been through increases in fibrillar proteins (Dickerson and Widdowson, 1960).

As stages of gestation progressed, muscle weights increased linearly ($P<.05$), indicating that either normal gilt growth or pregnancy-induced growth was causing increased deposition of one or more muscle constituents. Total grams of sarcoplasmic proteins increased ($P<.05$) with stage of gestation, but can explain only a small part of total muscle growth. Fibrillar proteins (percentage or total grams) were not significantly affected by stage of gestation although there was a tendency toward increased total g as pregnancy progressed.

Crude protein expressed as a percentage of wet tissue weight was affected by a significant protein level x stage of gestation interaction and examination on a within protein level basis yielded no significant differences due to stage of gestation. Crude protein percentages of 16 to 18% of wet weight were within the range of values obtained for pig muscle by Gilbreath and Trout (1973).

Tables XI through XV show tissue protein data expressed on a dry, ether extracted basis. Table XI indicates that both muscle and uterine dry, ether extract percentages were affected by significant protein level by stage of gestation interaction which resulted in tissue protein data affected

by similar interactions when expressed on a dry, ether extracted basis. Tables XII through XV show this same data expressed on a within protein level basis. Numbers of observations per subclass cell were inadequate to establish any stage of gestation effects on a within protein level basis. This data emphasizes the problems associated with use of tissue levels of proteins in evaluating dietary adequacy as reviewed by Waterlow (1969) and favors the use of total g of protein per muscle or organ.

Since protein intake did not significantly affect nitrogen retention, it is not surprising that protein intake effects on muscle protein were minimal. Stage of gestation had a significant quadratic effect ($P<.05$) on nitrogen retention which is difficult to explain. As gestation progressed, retention declined from 7.3 to -16.3 g/day while muscle weights and protein levels generally increased. It is apparent that either protein was translocated to these muscles from other tissues (viscera or other muscles) or nitrogen balance was underestimated. The latter explanation seems doubtful since nitrogen balance is a relatively routine procedure which has an inherent tendency to overestimate rather than underestimate retention.

Uterine Proteins

As was the case with muscle weights, uterine weights (Table XVI) were not significantly influenced by protein intake. Fibrillar and sarcoplasmic proteins, expressed as

percentages of wet tissues, increased linearly ($P<.05$) with increasing protein intake. When multiplied by uterine weights to give total g of proteins per gravid uterus, non-significant trends show increasing fibrillar and sarcoplasmic proteins with additional dietary protein. This may reflect the deposition of these two protein fractions in the fetal tissues which has been studied by Dickerson and Widdowson(1960).

Considering the lack of effect of protein intake on uterine crude protein (percentage and total g) and other non-significant trends, it may be concluded that protein intake in this experiment did not substantially affect growth in the developing fetuses. As in muscle data, the failure of protein intake to influence nitrogen retention would lead us to expect negligible effects on uterine proteins. Leathem et al. (1968) used severe protein restrictions (0% versus 20% casein) to demonstrate a reduction in total uterine weights and total uterine protein at day 13 of pregnancy in rats. Svajgr et al. (1972) reported lighter uterine weights in first litter sows fed low protein levels (5% crude protein).

The growth curve of the uterus and its contents appeared to be linear ($P<.01$) from 30 to 90 days of gestation as previously illustrated by Hafez and Dyer (1969). This growth curve was reflected by linear ($P<.01$) increases in percentage and total g of fibrillar proteins, total sarcoplasmic protein and total crude protein. The linear

nature of these curves does not support the calculations of Moustgaard (1962) which suggest that nitrogen retention for intrauterine deposition would rise exponentially as gestation progressed. However, two factors must be remembered. Firstly, some 25 days remained in this experiment before gestation was completed and secondly, it has been proposed (Chapter III) that energy was limiting in this trial. Such a limitation would certainly diminish the possibility of an exponential increase in protein deposition in the uterus.

It is important to note that as nitrogen retention decreased quadratically ($P<.05$), synthesis of proteins in the gravid uterus continued. This emphasizes the high priority of the uterus for available protein (Pond, 1973) and may also indicate an elevated protein turnover rate resulting in greater losses of nitrogen from gilts in this experiment. The source of protein for uterine growth as pregnancy progressed was not evidenced by tissue data or nitrogen balance data in this experiment. Tissues other than semitendinosus and biceps femoris muscles and liver may have contributed to the 809 g of crude protein present in the gravid uterus at day 90 of gestation. Nitrogen balance at this stage was -16.3 g/day suggesting that considerably more protein was being catabolized.

Liver and Uterine Fluids

As shown in Table XVII, liver weights and total g of crude protein in the liver responded in a significant quad-

ratic manner to increasing dietary protein while crude protein percentage (wet basis) responded in a linear ($P<.01$) manner. Both liver weight and total g of crude protein apparently increased from the 8 to 14% crude protein diets then plateaued or decreased slightly at higher protein levels. Allison et al. (1964) have reported that liver size in rats is increased as casein intake increases but plateaus at a nitrogen intake of approximately 3 g/kg of body weight/day.

Protein intake did not significantly influence the volume, crude protein concentration or total crude protein in the fluids which nourish and cushion the fetuses and are, in part, excretory products of the fetuses. The extent of nourishment provided by these fluids is not well documented.

Stage of gestation had no apparent effect on liver weights, crude protein percentage or total crude protein. The liver is widely recognized as the most dynamic protein storage organ (Kosterlitz and Campbell, 1945; Allsion and Wannemacher, 1965). Therefore, if any appreciable storage and translocation of protein were occuring, it is logical to expect changes in liver weights and crude protein. In this experiment the liver provides no explanation as to the source of protein deposited in the gravid uterus.

Uterine fluid volume, crude protein concentration, and total crude protein responded to progressing stage of gestation in various quadratic ($P<.01$) manners. Fluid volume increased from 2.47 liters at 30 days of gestation to 4.82

liters at mid gestation, then declined to 1.82 liters at 90 days. Hafez and Dyer (1969) illustrate fluid accumulation until about 90 days of gestation, then a plateau or slight drop in volume near parturition. Gilts in this experiment accumulated much more fluid in mid gestation than could be expected from their data. Fluid crude protein concentration increased throughout gestation while total g of crude protein increased to mid gestation, then plateaued or declined slightly at 90 days of pregnancy.

In general, increasing crude protein intake in experimental gilts caused small increases in total muscle fibrillar protein and crude protein, in wet tissue percentage of uterine fibrillar and sarcoplasmic proteins, and in liver weights and total crude protein to a plateau level. The main effects of progressing stage of gestation were increased muscle weights and total sarcoplasmic protein, increased uterine weights, total sarcoplasmic, fibrillar and crude proteins and increased uterine fluid crude protein to a plateau level. Therefore, tissue protein levels do not support a scheme of protein storage and translocation in this experiment.

Muscle DNA and RNA

Table XVIII presents muscle DNA and RNA data expressed on a dry ether, extracted basis. Since this extraction was a part of the nucleic acid analysis and protein level x stage of gestation interactions were not indicated, values

are expressed on this basis. Muscle DNA expressed as concentration, total g per muscle or mg/g of sarcoplasmic plus fibrillar protein was not significantly affected by protein intake or stage of gestation. This data indicates that there was not an increase in nuclei numbers of muscles as would be expected if hyperplasic growth were occuring (Stromer et al., 1974). A trend toward a decline in DNA per unit of protein over stages of gestation indicates that muscle growth was by hypertrophy, not hyperplasia. Winick and Noble (1965) defined three phases of growth for the rat from conception to adulthood. Phase one involved hyperplasia, phase two combined hyperplasia and hypertrophy and the third phase involved only hypertrophy. Martin et al. (1974) and Robinson (1969) suggested that in the pig, some degree of hyperplasia or increase in muscle nuclei numbers can continue long after birth (3 to 6 months). In this experiment nutritional factors (protein plus energy) may not have been sufficient to stimulate increases in nucleus-cytoplasm units. The current theory involving muscle nuclei numbers is based on the proposal that satellite cells (small, mono-nucleated cells) which lie on the periphery of muscle cells can fuse with those muscle cells to add nuclei. This mechanism allows expansion of potential cell mass even after nuclear division has ceased within the cell (Stromer et al., 1974).

Muscle RNA concentration and total g of RNA were not significantly affected by protein intake. There was a

trend ($P<.10$) toward increasing muscle RNA concentration as pregnancy progressed which became a significant linear increase when expressed as total muscle RNA. This indicates that protein synthetic activity in these muscles was increasing, possibly to increase muscle protein stores. The ratio of RNA to DNA also increased with stage of gestation supporting these previous statements. It is suggested that the muscles were increasing their protein synthetic capacity under the influence of increased substrate (free amino acids) or anabolic hormones (Bergen, 1974).

Uterine DNA and RNA

Table XIX presents nucleic acid data for uterine tissues on a dry, ether extracted basis. Concentration of DNA (mg/g) and total g of DNA per gravid uterus were highly variable and differences among protein intake groups were not significant. This suggests that cellular growth in the gravid uterus did not respond to increasing protein intake of the gilt. Since the low protein diet appeared adequate for maximal hyperplasia, some other nutrient (such as energy) may have been inadequate to allow a response to elevated protein intake.

Uterine DNA per unit of sarcoplasmic plus fibrillar protein was not significantly influenced by protein level, although values ranged from 59.51 mg/g for the 20% diet to 76.45 mg/g for the 14% diet. This measurement was calculated to indicate the degree of hypertrophy or protein

deposition per nucleus; however, a consistent trend is not apparent with respect to protein intake effects on this measure of cellular growth.

Stage of gestation did not alter uterine DNA concentrations but total g of DNA increased linearly ($P<.01$) from 30 to 90 days of gestation implying that hyperplasia or increases in nuclei numbers continued at a similar rate throughout this part of gestation.

Uterine RNA concentration was not responsive to protein intake or stage of gestation and total g of RNA did not respond to protein intake. Leathem et al. (1968) outlined the effects of protein nutrition on the biochemical composition of the rat and mouse uterus. These authors cited decreases in total RNA per uterus due to severe protein deprivation while concentrations of RNA were not changed. Increases in uterine RNA were significantly linear as gestation progressed in this experiment. These increases in RNA were not proportionate to increases in nuclei as indicated by RNA to DNA ratios of 2.85, 3.68, and 3.04 for 30, 60, and 90 days, respectively.

In general, increasing protein intake in this experiment had little or no influence on muscle and gravid uterine levels of DNA and RNA. Naismith and Morgan (1974) have reported increased fetal and placental weight, total uterine protein and total uterine DNA in pregnant rats when fed supplemental protein. The failure to achieve such a response in these gilts has tentatively been attributed to

inadequate energy allowances. The effects of stage of gestation on DNA and RNA have been described as linear increases in total muscle RNA and total uterine RNA and DNA. These measurements indicate the nature of protein synthetic activity (RNA) and hyperplasia or increases in nuclei numbers (DNA).

TABLE IX

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	
Total (Corrected)	36	
Protein Level	3	
Linear		1
Quadratic		1
Cubic		1
Stage of Gestation	2	
Linear		1
Quadratic		1
Protein Level x Stage of Gestation	6	
Animals/Protein Level x Stage of Gestation	25	

TABLE X

MUSCLE PROTEINS FROM GILTS FED GRADED LEVELS OF PROTEIN AT THREE STAGES OF GESTATION[a]

Item	Protein Level				Stage of Gestation		
	8%	14%	17%	20%	30	60	90
Muscle weights[b] (g)	2660±103	2830±95	2920±107	2850±107	2660±91	2820±91	2960±77[c]
Fibrillar protein (%)	5.53±.21	5.51±.21	6.06±.21	5.94±.24	5.79±.20	5.79±.20	5.70±.17
Fibrillar protein (g)	147±9.0	155±8.2	177±8.2	170±9.3[c]	156±7.9	164±7.9	168±6.7
Sarcoplasmic protein (%)	2.84±.11	3.02±.10	2.87±.10	2.92±.11	2.79±.10	2.99±.10	2.95±.08
Sarcoplasmic protein (g)	75.2±4.3	95.4±4.0	84.1±4.0	83.6±4.5	74.2±3.8	84.8±3.8	87.3±3.3[c]
Crude protein[d] (%)	16.4±.35	18.0±.33	17.1±.33	17.6±.37	17.3±.31	18.0±.31	16.5±.27
Crude protein (g)	436±21.5	510±19.8	499±19.8	503±22.3[c]	461±19.0	511±19.0	489±16.1

[a]Means ± standard errors on a wet tissue basis.

[b]Semitendinosus and biceps femoris muscles of left ham.

[c]Linear effect significant ($P<.05$).

[d]Significant protein level x stage of gestation interaction ($P<.05$).

TABLE XI

MUSCLE AND UTERINE TISSUE DATA EXPRESSED ON A DRY, ETHER EXTRACTED BASIS[a]

Item	Protein Level				Stage of Gestation		
	8%	14%	17%	20%	30	60	90
Muscle DEET[b,c]	27.2±.62	33.4±.57	28.6±.57	30.4±.65	31.2±.55	29.1±.55	29.4±.47
Muscle crude protein[c]	60.3±.75	54.6±.69	59.9±.69	59.0±.78	55.7±.66	62.1±.66	57.5±.56
Muscle fibrillar protein[c]	20.5±.94	16.6±.87	21.3±.87	19.9±.98	18.7±.83	20.2±.83	19.9±.71
Muscle sarcoplasmic protein[c]	10.5±.41	9.1±.38	10.1±.38	9.8±.43	9.0±.36	10.4±.36	10.2±.31
Uterine DEET[b,c]	16.2±.52	20.4±.48	20.9±.48	16.6±.54	20.5±.46	17.8±.46	17.3±.39
Uterine crude protein[c]	48.2±.63	41.5±.58	40.4±.58	48.8±.65	43.2±.55	42.9±.55	48.1±.47
Uterine fibrillar protein[c]	5.4±.35	4.9±.33	5.0±.33	6.6±.37	4.4±.31	5.4±.31	6.7±.27
Uterine sarcoplasmic protein[c]	12.4±.52	9.5±.48	11.3±.48	13.6±.54	10.9±.46	12.4±.46	11.8±.39

[a]Values given in percentages ± standard errors.

[b]DEET defined as dry, ether extracted tissue.

[c]Protein level x stage of gestation interaction significant ($P<.05$).

TABLE XII

MUSCLE AND UTERINE TISSUE DATA EXPRESSED ON A DRY, ETHER EXTRACTED BASIS FOR GILTS FED THE 8% DIET[a]

Item	Stage of Gestation		
	30	60	90
Muscle DEET[b]	28.7±1.27	26.3±1.04	26.6±.90
Muscle crude protein	56.2±1.52	62.8±1.24	62.0±1.08
Muscle fibrillar protein	19.6±1.92	21.6±1.57	20.1±1.36
Muscle sarcoplasmic protein	9.7±.84	11.0±.68	10.7±.59
Uterine DEET[b]	19.1±1.06	13.9±.87	15.7±.75
Uterine crude protein	40.7±1.27	51.0±1.04	52.8±.90
Uterine fibrillar protein	4.1±.72	5.3±.59	7.0±.51
Uterine sarcoplasmic protein	10.2±1.06	14.9±.86	12.0±.75

[a]Values given in percentages ± standard errors.

[b]DEET defined as dry, ether extracted tissue.

TABLE XIII

MUSCLE AND UTERINE TISSUE DATA EXPRESSED ON A DRY, ETHER EXTRACTED BASIS FOR GILTS FED THE 14% DIET[a]

Item	Stage of Gestation		
	30	60	90
Muscle DEET[b]	30.8±1.04	32.8±1.04	36.6±.90
Muscle crude protein	60.2±1.24	60.7±1.24	42.8±1.08
Muscle fibrillar protein	18.6±1.57	15.7±1.57	15.5±1.36
Muscle sarcoplasmic protein	9.6±.68	9.4±.68	8.3±.59
Uterine DEET[b]	18.1±.87	21.5±.87	21.8±.75
Uterine crude protein	48.3±1.04	38.9±1.04	37.3±.90
Uterine fibrillar protein	5.5±.59	4.2±.59	4.9±.51
Uterine sarcoplasmic protein	11.6±.86	9.2±.86	7.7±.75

[a]Values given in percentages ± standard errors.

[b]DEET defined as dry, ether extracted tissue.

TABLE XIV

MUSCLE AND UTERINE TISSUE DATA EXPRESSED ON A DRY, ETHER EXTRACTED BASIS FOR GILTS FED THE 17% DIET[a]

Item	Stage of Gestation		
	30	60	90
Muscle DEET[b]	30.6±1.04	26.9±1.04	28.5±.90
Muscle crude protein	57.3±1.24	64.2±1.24	58.1±1.08
Muscle fibrillar protein	19.6±1.57	24.4±1.57	20.0±1.36
Muscle sarcoplasmic protein	9.1±.68	10.9±.68	10.3±.59
Uterine DEET[b]	26.2±.87	20.6±.87	15.7±.75
Uterine crude protein	34.4±1.04	35.4±1.04	51.4±.90
Uterine fibrillar protein	2.7±.59	4.6±.59	7.6±.51
Uterine sarcoplasmic protein	8.7±.86	10.7±.86	14.6±.75

[a]Values given in percentages ± standard errors.

[b]DEET defined as dry, ether extracted tissue.

TABLE XV

MUSCLE AND UTERINE TISSUE DATA EXPRESSED ON A DRY, ETHER EXTRACTED BASIS FOR GILTS FED THE 20% DIET[a]

Item	Stage of Gestation		
	30	60	90
Muscle DEET[b]	34.7±1.04	30.5±1.27	25.9±1.04
Muscle crude protein	49.2±1.24	60.8±1.52	67.2±1.24
Muscle fibrillar protein	17.0±1.57	19.0±1.92	23.8±1.57
Muscle sarcoplasmic protein	7.7±.68	10.1±.84	11.6±.68
Uterine DEET[b]	18.6±.87	15.0±1.06	16.1±.87
Uterine crude protein	49.2±1.04	46.2±1.27	51.0±1.04
Uterine fibrillar protein	5.0±.59	7.4±.72	7.4±.59
Uterine sarcoplasmic protein	12.9±.86	14.7±1.06	13.1±.86

[a]Values given in percentages ± standard errors.

[b]DEET defined as dry, ether extracted tissue.

TABLE XVI

UTERINE PROTEINS FROM GILTS FED GRADED LEVELS OF PROTEIN AT THREE STAGES OF GESTATION[a]

Item	Protein Level				Stage of Gestation		
	8%	14%	17%	20%	30	60	90
Uterine weights (g)	6190±854	6240±785	5860±785	6480±886	2140±753	5900±753	10500±640[b]
Fibrillar protein (%)	.86±.06	.99±.06	.95±.06	1.08±.07[c]	.86±.06	.92±.06	1.14±.05[b]
Fibrillar protein (g)	58.1±10.3	63.1±9.5	60.3±9.5	74.6±10.7	18.7±9.1	54.2±9.1	119.1±7.7[b]
Sarcoplasmic protein (%)	1.97±.08	1.98±.08	2.26±.08	2.23±.09[c]	2.19±.07	2.11±.07	2.03±.06
Sarcoplasmic protein (g)	121±16.3	119±15.0	130±15.0	139±16.9	47.8±14.4	124±14.4	211±12.2[b]
Crude protein (%)	7.72±.23	8.39±.21	8.13±.21	8.11±.24	8.68±.21	7.40±.21	8.18±.18[d]
Crude protein (g)	433±76.3	513±70.2	458±70.2	505±79.2	188±67.3	435±67.3	809±57.2[b]

[a]Means ± standard errors on a wet tissue basis.
[b]Linear effect significant ($P<.01$).
[c]Linear effect significant ($P<.05$).
[d]Quadratic effect significant ($P<.01$).

TABLE XVII

LIVER AND UTERINE FLUID MEASUREMENTS AND CRUDE PROTEIN VALUES[a]

Item	Protein Level				Stage of Gestation		
	8%	14%	17%	20%	30	60	90
Liver weights (g)	1370±49	1510.1±45	1430±45	1350±51[b]	1380±43	1400±43	1470±37
Liver crude protein (%)	17.1±.29	18.4±.27	18.4±.27	18.9±.30[c]	18.5±.26	18.1±.26	18.1±.22
Liver crude protein (g)	234±8.7	278±8.0	264±8.0	256±9.0[d]	256±7.6	252±7.6	265±6.5
Uterine fluid volume (liters)	3.76±.52	2.44±.48	3.40±.48	2.55±.54	2.47±.46	4.82±.46	1.82±.39[d]
Fluid crude protein (mg/ml)	8.8±.92	11.0±.85	10.5±.85	11.1±.95	4.2±.81	8.3±.81	18.6±.69[d]
Fluid crude protein (g)	27.6±3.7	25.9±3.4	26.6±3.4	23.5±3.9	9.1±3.3	35.5±3.3	33.2±2.8[d]

[a]Means ± standard errors on a wet tissue basis.

[b]Quadratic effect significant ($P<.05$).

[c]Linear effect significant ($P<.01$) and liver crude protein percentage expressed on wet tissue basis.

[d]Quadratic effect significant ($P<.01$).

TABLE XVIII

MUSCLE DNA AND RNA LEVELS AS AFFECTED BY PROTEIN INTAKE AND STAGE OF GESTATION IN GILTS[a]

Item	Protein Level				Stage of Gestation		
	8%	14%	17%	20%	30	60	90
Muscle DNA (mg/g)	6.87±.44	6.83±.40	6.70±.40	7.14±.45	7.08±.39	6.99±.39	6.58±.33
Muscle RNA (mg/g)	26.60±1.51	23.63±1.39	25.67±1.39	25.39±1.33	23.60±1.33	25.22±1.33	27.15±1.13[e]
Muscle RNA/DNA	4.00±.28	3.57±.25	3.88±.25	3.69±.29	3.39±.24	3.71±.24	4.25±.21[c]
Muscle DNA/Protein[b]	22.37±1.25	26.59±1.41	21.72±1.21	24.41±1.45	25.69±1.27	23.18±1.18	22.51±1.01
Total muscle DNA (g)	5.02±.42	6.42±.47	5.59±.41	6.17±.49	5.90±.42	5.76±.40	5.71±.34
Total muscle RNA (g)	19.51±1.39	22.37±1.57	21.53±1.35	22.06±1.61	19.77±1.41	20.78±1.31	23.56±1.13[d]

[a]Means ± standard error on dry, ether extracted basis.

[b]Expressed as mg DNA per g of sarcoplasmic plus fibrillar protein.

[c]Linear effect significant ($P<.05$).

[d]Linear effect significant ($P<.01$).

[e]Linear effect approached significance ($P<.10$).

TABLE XIX

UTERINE DNA AND RNA LEVELS AS AFFECTED BY PROTEIN INTAKE AND STAGE OF GESTATION IN GILTS[a]

Item	Protein Level				Stage of Gestation		
	8%	14%	17%	20%	30	60	90
Uterine DNA (mg/g)	12.04±1.53	11.11±1.41	11.73±1.41	12.18±1.59	12.40±1.35	10.63±1.35	12.26±1.15
Uterine RNA (mg/g)	33.02±3.41	31.82±3.14	33.83±3.14	36.15±3.54	31.37±3.01	35.82±3.01	33.93±2.56
Uterine RNA/DNA	2.96±.47	3.26±.44	3.17±.44	3.37±.49	2.85±.42	3.68±.42	3.04±.35
Uterine DNA/Protein[b]	69.42±8.27	76.45±9.58	76.32±9.50	59.51±8.77	85.01±9.22	62.61±7.98	66.39±6.62
Total uterine DNA (g)	11.50±2.42	15.52±2.81	14.01±2.86	12.27±2.57	5.32±2.70	11.21±2.34	21.99±1.94[c]
Total uterine RNA (g)	35.21±6.83	39.74±7.91	42.53±8.07	38.03±7.24	14.32±7.63	37.40±6.59	61.15±5.47[c]

[a]Means ± standard errors on dry, ether extracted basis.

[b]Expressed as mg DNA per g of sarcoplasmic plus fibrillar protein.

[c]Linear effect significant ($P<.01$).

CHAPTER V

CONCLUSIONS

Optimizing reproductive efficiency in the gilt is vital to the overall efficiency of pork production. To optimize this efficiency, we must integrate the best genetic material with an ideal environment. A major factor in such an environment is the provision of proper nutrients for maintenance and growth of the gilt, for fetal growth, and for subsequent lactation. It may be necessary to subdivide the reproductive cycle into several stages with specific feeding regimes for prebreeding development; early, mid, and late gestation; and lactation to insure meeting the specific requirements of the gilt while maintaining economy of production. This study attempted to establish the effects of protein intake and stage of gestation on various parameters which should provide a broader understanding of the protein requirements of the gestating gilt.

Protein intake of the gilt did not significantly affect reproductive performance as measured by number of corpora lutea, number of live embryos, survival rate or embryo lengths. This concurs with other data found in the literature. The large degree of variation in reproductive performance criteria of swine makes it difficult to statisti-

cally document differences in mean performance which appear to be of practical importance. Careful studies with large numbers of gilts are needed to clearly establish the effects of protein on reproductive performance.

Total body weight gain and average daily gain were not significantly affected by protein intake. This suggests that the lowest feed intake level, calculated as 147 g of crude protein and 6323 kcal of digestible energy, was sufficient for body weight gain or that energy was inadequate to support a weight gain response to increasing protein intake. Further work is needed to define the energy intake necessary for optimal use of dietary protein. Nitrogen retention in these trials was complicated by trial interactions. In trial 1, nitrogen retention increased as protein intake increased, a result that was anticipated due to previous studies. But trial 2 resulted in extremely low retention values which did not respond to increasing protein intake. Three factors are offered in explanation of trial 2 balance data. Trial 2 was conducted in the colder season of the year when maintenance energy requirements of the gilt are highest. If the energy supplied was inadequate, dietary and tissue proteins would be catabolized for energy purposes with subsequent loss of nitrogen. Another factor is that gilts in trial 2 were approximately 20 kg lighter than gilts in trial 1. This may indicate that tissue stores of fat and protein in trial 2 gilts were inadequate to meet elevated gestation requirements. A third factor which may explain

some of the trial differences in nitrogen retention is the failure to provide crude protein intakes as in trial 1. Trial 2 crude protein values were approximately 88% of intended levels.

Tissue data was derived from gilts in trial 2 only. Since the high levels of nitrogen retention were not achieved as in previous trials, corresponding increases in muscle proteins could not be expected. However, total muscle fibrillar and crude protein increased as protein intake was increased. Since total muscle DNA was not influenced by protein intake, the increases in fibrillar and crude protein suggest that growth of muscle was by hypertrophy. The magnitude of increases in muscle proteins does not appear sufficient to propose a protein storage scheme. If nitrogen retention had been comparable to that in previous trials, important conclusions may have been possible as to the extent of protein that a gestating gilt can store for use in late gestation or lactation.

In this study, various performance criteria were evaluated before breeding and during early, mid, and late gestation. Conception increased nitrogen retention by 4.1 g/day in trial 1, but no effect was seen in trial 2. Stage of gestation also influenced nitrogen retention in trial 1, with similar retention in early and late gestation and lower values at mid gestation. This data suggests a complex control system possibly mediated by hormonal factors. In trial 2, stage of gestation effects on nitrogen retention

were shown as a quadratic decrease to -16.3 g/day in late gestation. Obviously, whatever system provides control over nitrogen retention was entirely changed from trial 1 gilts. In a lactation period following such nitrogen losses, milk production would likely be impaired.

As gestation progressed from 30 to 90 days, muscles increased in weight and total sarcoplasmic proteins and protein synthetic activity increased as measured by total muscle RNA. It is apparent that the gilts muscular system was prepared to synthesize proteins; however, amino acid pools may have been inadequate to supply precursors for extensive storage protein synthesis. The growth of the uterus and its contents was linear over stages of gestation. Gravid uterine tissue weight; fibrillar, sarcoplasmic and crude protein values; and DNA and RNA each responded in a positive linear manner to progressing gestation. Tissue data suggests the hyperplasic nature of fetal growth from 30 to 90 days of gestation.

In conclusion, the intent of this experiment was to achieve elevated nitrogen retention with graded levels of protein intake in the gilt, then to evaluate the extent, chemical nature and physiological importance of that high nitrogen retention. Since the first part of this objective was not met in trial 2, tissue data are inconclusive. Further research is indicated from this failure to observe expected nitrogen retention levels.

SELECTED BIBLIOGRAPHY

Allison, J.B., and R.W. Wannemacher, Jr. 1965. The concept and significance of labile and over-all protein reserves of the body. Amer. J. Clin. Nutr. 16:445.

Allison, J.B., and R.W. Wannemacher, Jr. and W.B. Banks, Jr. 1963. Influence of dietary proteins on protein biosynthesis in various tissues. Fed. Proc. 22:1126.

Allison, J.B., R.W. Wannemacher, Jr., W.L. Banks, Jr., and W.H. Wunner. 1964. The magnitude and significance of the protein reserves in rats fed at various levels of Nitrogen. J. Nutr. 84:383.

A.O.A.C. 1960. Official Methods of Analysis. Association of Official Agriculture Chemists, Washington, D.C.

Baker, D.H., D.E. Becker, A.H. Jensen, and B.G. Harmon. 1970. Reproductive performance and progeny development in swine as influenced by protein restriction during various portions of gestation. J. Animal Sc. 31:526.

Baker, D.H., D.E. Becker, H.W. Norton, C.E. Sasse, A.H. Jensen, and B.G. Harmon. 1969. Reproductive performance and progeny development in swine as influenced by feed intake during pregnancy. J. Nutr. 97:489.

Bergen, W.G. 1974. Protein synthesis in animal models. J. Animal Sci. 38:1079.

Calvez, A.E., T.D. Tanksley, Jr., and J.H. Hesby. 1973. Performance of swine fed fortified sorghum grain during gestation. J. Animal Sci. 37:275 (Abstr.).

Campbell, R.M., I.R. Innes, and H.W. Kosterlitz. 1953. Some dietary and hormonal effects on maternal, foetal, and placental weights in the rat. J. Endo. 9:68.

Campbell, R.M., and H.W. Kosterlitz. 1952. The absence of dietary effects on the DNA content of liver nuclei of the adult rat. Science 115:84.

Clawson, A.J., H.L. Rickards, G. Matrone, and R.E. Barrick. 1963. Influence of level of total nutrient and protein intake on reproductive performance in swine. J.

Animal Sci. 22:662.

Degeeter, M.J., V.M. Hays, D.D. Kratzer, and G.L. Cromwell. 1972. Reproductive performance of gilts fed diets low in protein during gestation and lactation. J. Animal Sci. 35:772.

Dickerson, J.W.T. and R.A. McCance. 1960. Severe undernutrition in growing and adult animals. 3. Avian skeletal muscle. Br. J. Nutr. 14:331.

Dickerson, J.W.T. and E.M. Widdowson. 1960. Chemical changes in muscle during development. Biochem. J. 74:247.

Duncan, D.L. and G.A. Lodge. 1960. Diet in relation to reproduction and the viability of the young. Part III. Pigs. Technical Communication No. 21. Commonwealth Bureau of Animal Nutrition, Aberdeen, Scotland.

Elsley, F.W.H., D.M. Anderson, I. McDonald, R.M. Mac Pherson, and R.Smart. 1966. A comparison of the liveweight changes, nitrogen retention and carcass composition of pregnant and non-pregnant gilts. Anim. Prod. 8:391.

Enesco, M. and C.P. LeBlond. 1962. Increase in cell number as a factor in the growth of the organs and tissues of the young male rat. J. Emb. and Expt. Morph. 10:530.

Enesco, M. and D. Puddy. 1964. Increase in the number of nuclei and weight in skeletal muscle of rats of various ages. Am. J. Anat. 114:235.

Escoubas, J.R. 1973. Quantitative changes in total nitrogen and its components in bovine longissimus muscle during growth and development. M.S. thesis, Oklahoma State University, Stillwater, OK.

Evans, R.E. 1929. Protein and mineral metabolism in pregnant sows on a normal or high calcium diet compared with a calcium-deficient diet. J. Agri. Sci. 19:752.

Frobish, L.T., V.C. Speer, and V.W. Hays. 1966. Effect of protein and energy intake on reproductive performance in swine. J. Animal Sci. 25:729.

Gilbreath, R.L. and J.R. Trout. 1973. Effects of early postnatal dietary protein restriction and repletion on porcine muscle growth and composition. J. Nutr. 103:1637.

Goldberg, A.L. 1968. Protein synthesis during work-induced growth of skeletal muscle. J. Cell Biol. 36:653.

Goldberg, A.L. 1969. Protein turnover in skeletal muscle. J. Biol. Chemistry 244:3217.

Goldberg, A.L. and J.F. Dice. 1974. Intracellular protein degradation in mammalian and bacterial cells. Annu. Rev. Biochem. 43:835.

Gordon, E.E., K. Kowalski and M. Fritts. 1966. Muscle proteins and DNA in rat quadriceps furing growth. Am. J. Physio. 210:1033

Hafez, E.S.E. and I.A. Dyer. 1969. Animal growth and nutrition. Lea and Febiger, Philadelphia.

Hanly, S. 1961. Prenatal mortality in farm animals. J. Reprod. Fert. 2:182.

Harvey, W.R. 1960. Least squares analysis of data with unequal subclass numbers. U.S.D.A., A.R.S. Bul. 20-8.

Haught, D.G., T.D. Tanksley, Jr., J.H. Hesby, and E.J. Gregg. 1974. Protein level and source on swine reproduction and progeny growth. J. Anim. Sci. 39:183 (Abstr.).

Hawton, J.D. and R.J. Meade. 1971. Influence on quantity and quality of protein fed the gravid female on reproductive performance and development of offspring in swine. J. Animal Sci. 32:88.

Heap, F.C. and G.A. Lodge. 1967. Changes in body composition of the sow during pregnancy. Anim. Prod. 9:237.

Helander, E.A.S. 1957. On quantitative muscle protein determination. Acta Physiol. Scand. 41:Suppl. 141.

Hesby, J.H., J.H. Conrad, M.P. Plumlee, and R.B. Harrington. 1971. Nitrogen balance and serum protein response of gestating swine fed opaque-2 corn, normal corn and corn-soybean diets. J. Animal Sci. 31:481.

Hesby, J.H., J.H. Conrad, M.P. Plumlee and T.G. Martin. 1970. Opaque-2 corn, normal corn and corn-soybean meal gestation diets for swine reproduction. J. Animal Sci. 30:474.

Hesby, J.H., D.G. Haught, and T.D. Tanksley, Jr. 1973. Feeding interval and protein levels on gravid gilt nitrogen balance. J. Animal Sci. 37:282. (Abstr.).

Hines, R.H., G.L. Allee, and B.A. Koch. 1973. Fortified milo for gestating sows. J. Animal Sci. 37:282 (Abstr.).

Howarth, R.E. 1972. RNA content and protein synthesis in skeletal muscles of young rats. Effects of protein deficiency. Can. J. Physiol. Pharm. 50:59.

Jones, R.D. 1973. The effects of dietary protein levels on growth, nitrogen balance and reproductive performance in gilts. M.S. thesis, Oklahoma State University, Stillwater, OK.

Jones, R.D. and C.V. Maxwell. 1974. Effect of protein level on growth, nitrogen balance and reproductive performance in gilts. J. Animal Sci. 39:1067.

Kline, R.D., L.L. Anderson, and R.M. Melampy. 1972. Nitrogen retention in pigs during the estrous cycle or pregnancy and after hysterectomy or ovarectomy. J. Animal Sci. 35:585.

Kosterlitz, H.W. and R.M. Campbell. 1945. The storage of protein in the adult animal. Nutr. Abstr. Rev. 15:1.

Lash, J.W., H. Holtzer, and H. Swift. 1957. Regeneration of mature skeletal muscle. Anat. Rec. 128:679.

Leathem, J.H., R. Oslapas, and C.J. Fisher. 1968. Protein nutrition and the biochemical composition of the uterus. Fertil. and Steril. 19:206.

Low, R.B. and A.L. Goldberg. 1973. Non uniform rates of turnover of myofibrillar proteins in rat diaphragm. J. Cell Biol. 56:590.

Manchester, K.L. 1970. Sites of hormonal regulation of protein metabolism. In H.M. Munro (Ed.). Mammalian Protein Metabolism. Vol. IV. Academic Press, New York.

Manchester, K.L. 1972. Effect of insulin on protein synthesis. Diabetes 21:447.

Martin, R.J., M. Ezekwe, J.H. Herbein, G.W. Sherritt, J.L. Gobble, and J.H. Ziegler. 1974. Effects of neonatal nutrition experiences on growth and development of the pig. J. Animal Sci. 39:521.

Mendes, C.B. and J.C. Waterlow. 1958. The effect of a low protein diet and of refeeding on the composition of liver and muscle in the weanling rat. Br. J. Nutr. 12:74.

Miller, G.M., D.E. Becker, A.H. Jensen, B.G. Harmon, and H.W. Norton. 1969. Effect of protein intake on nitrogen retention by swine during late pregnancy. J. Animal Sci. 28:204.

Mitchell, H.H., W.E. Carroll, T.S. Hamilton, and G.E. Hunt. 1931. Food requirements of pregnancy in swine. Univ. Ill. Agri. Exp. Sta. Bull. 375.

Moustgaard, J. 1962. Foetal nutrition in the pig. In J.T. Morgan and D. Lewis (Ed.). Nutrition of Pigs and Poultry. Butterworths, London.

Munro, H.N. 1964. General aspects of the regulation of protein metabolism by diet and by hormones. In H.N. Munro (Ed.). Mammalian Protein Metabolism. Vol I. Academic Press, New York.

Munro, H.N. and A. Fleck. 1969. Analysis of tissues and body fluids for nitrogenous constituents. In H.N. Munro (Ed.). Mammalian Protein Metabolism. Vol. III. Academic Press, New York.

Naismith, D.J. 1973. Adaptations in the metabolism of protein during pregnancy and their nutritional implications. Nutr. Rep. Int. 7:383.

Naismith, D.J. and B.L.G. Morgan. 1974. The effect of protein supplementation in early pregnancy on the growth of the rat foetus and placenta. Proc. Nutr. Soc. 33:58 (Abstr.).

Newton, W.H. 1956. In Marshall's Physiology of Reproduction. (3rd Ed.). Longmans, Green, London.

N.R.C. 1973. Nutrient Requirements of Domestic Animals, No. 2. Nutrient requirements of swine. National Academy of Science - National Research Council, Washington, D.C.

Polge, C., L.E.A. Rowson, and M.C. Chang. 1966. The effect of reducing the number of embryos during early stages of gestation on the maintenance of pregnancy in the pig. J. Reprod. Fert. 12:395.

Pollister, A.W., H. Swift . Alfert. 1951. Studies on the desoxypentose nucleic acid content of the animal nuclei. J. Cell and Comp. Physio. 38:101.

Pond, W.G. 1973. Influence of maternal protein and energy nutrition during gestation on progeny performance in swine. J. Animal Sci. 36:175.

Pond, W.G., L.D. VanVleck, E.F. Walker, Jr., C.F. Eisenhard, and J.R. O'Connor. 1969. Changes in body weight and composition of adult non gravid female rats deprived of dietary protein. J. Nutr. 97:343.

Pond, W.G., W.C. Wagner, J.A. Dunn, and E.F. Walker, Jr. 1968. Reproduction and early postnatal growth of progeny in swine fed a protein-free diet during gestation. J. Nutr. 94:309.

Pond, W.G., D.N. Strachan, Y.N. Sinha, E.F. Walker, Jr., J.A. Dunn and R.H. Barnes. 1969. Effect of protein deprivation of swine during all or part of gestation on birth weight, postnatal growth rate and nucleic acid content of brain and muscle of progeny. J. Nutr. 99:61.

Poo, L.J., W. Lew, D.D. Lee and T. Addis. 1940. Protein anabolism in the organs and tissues of pregnant rats at different levels of protein consumption. J. Nutr. 19:505.

Rippel, R.H. 1967. Protein and amino acid nutrition of gravid swine. J. Animal Sci. 26:526.

Rippel, R.H., O.G. Rasmussen, A.H. Jensen, H.W. Norton, and D.E. Becker. 1965a Effect of level and source of protein on reproductive performance of swine. J. Animal Sci. 24:203.

Rippel, R.H., B.G. Harmon, A.H. Jensen, H.W. Norton and D.E. Becker. 1965b. Response of the gravid gilt to levels of protein as determined by nitrogen balance. J. Animal Sci. 24:209.

Robinson, D.S. 1952. Changes in the protein composition of chick muscle during development. Biochem J. 52:621.

Robinson, D.W. 1969. The cellular response to porcine skeletal muscle to prenatal and neonatal nutritional stress. Growth 33:231.

Salmon-Legagneur, E. and A. Rerat. 1962. Nutrition of the sow during pregnancy. In J.T. Morgan and D. Lewis (Ed.). Nutrition of Pigs and Poultry. Butterworths, London.

Shearer, I.T., N.B. Haynes, and D.B. Crighton. 1971. Peripheral steroid hormone levels and nitrogen retention in the gilt from prepuberty to parturition. J. Reprod. Fert. 27:491.

Strohman, R.C., B. Paterson, R. Fluch and A. Przybyla.

1974. Cell fusion and terminal differentiation of myogenic cells in culture. J. Animal Sci. 38:1103.

Stromer, M.H., D.E. Goll, R.B. Young, R.M. Robson, and F.C. Parrish, Jr. 1974. Ultrastructural features of skeletal muscle differentiation and development. J. Animal Sci. 38:1111.

Stroun, M., P. Charles, P. Anker, and S.R. Pelc. 1967. Metabolic DNA in heart and skeletal muscle and in the intestine of mice. Nat. 216:716.

Svajgr, A.J., D.L. Hammell, M.J. Degeeter, V.W. Hays, G.L. Cromwell, and R.H. Dutt. 1972. Reproductive performance of sows on a protein-restricted diet. J. Reprod. Fert. 30:455.

Swick, R.W. and H. Song. 1974. Turnover rates of various muscle proteins. J. Animal Sci. 38:1150.

Thompson, R.Y., F.C. Heagy, W.C. Hutchison, and J.N. Davidson. 1952. The deoxyribonucleic acid content of the rat cell nucleus and its use in expressing the results of tissue analysis with particular reference to the composition of liver tissue. Biochem. J. 53:460.

Trenkle, A. 1974. Hormonal and nutritional interrelationships and their effects on skeletal muscle. J. Animal Sci. 38:1142.

Tucker, H.A. and R.P. Reece. 1962. Nucleic acid estimates of mammary tissue and nuclei. Proc. Exp. Biol. and Med. 111:639.

Venable, J.H. 1966. Constant cell populations in normal, testosterone-deprived and testosterone-stimulated levator ani muscles. Am. J. Anat. 119:263.

Venable, J.H. 1966. Morphology of the cells of normal, testosterone-deprived and testosterone-stimulated levator ani muscles. Am. J. Anat. 119:271.

Wannemacher, R.W., Jr. and W.K. Cooper. 1970. Relationship between protein metabolism in muscle tissue and the concept of "protein reserves". In C.P. Bianchi and R. Hilt (Ed.). Protein Metabolism and Biological Function. Rutgers University Press.

Waterlow, J.C. 1969. The assessment of protein nutrition and metabolism in the whole animal with special reference to man. In H.N. Munro (Ed.). Mammalian Protein Metabolism. Vol III. Academic Press, New York.

Waterlow, J.C. and J.M.L. Stephen. 1966. Adaptation of the rat to a low-protein diet: the effect of a reduced protein intake on the pattern of incorporation of L-[^{14}C] lysine. Br. J. Nutr. 20:461.

Widdowson, E.M., J.W.T. Dickerson, and R.A. McCance. 1960. Severe undernutrition in growing and adult animals. 4. The impact of severe undernutrition on the chemical composition of the soft tissues of the pig. Br. J. Nutr. 14:457.

Winick, M. and A. Noble. 1965. Quantitative changes in DNA, RNA, and protein during prenatal and postnatal growth in the rat. Devel. Biol. 12:451.

Winick, M. and A. Noble. 1967. Cellular response in rat during malnutrition at various ages. J. Nutr. 89:300.

Young, V.R. 1974. Regulation of protein synthesis and skeletal muscle growth. J. Animal Sci. 38:1054.

VITA

Richard Don Jones

Candidate for the Degree of

Doctor of Philosophy

Thesis: INFLUENCE OF PROTEIN INTAKE AND STAGE OF GESTATION ON GROWTH, REPRODUCTIVE PERFORMANCE, NITROGEN BALANCE, AND TISSUE LEVELS OF PROTEINS, DNA, AND RNA IN GILTS.

Major Field: Animal Nutrition

Biographical:

Personal Data: Born in Pampa, Texas, January 26, 1947, the son of M.A. and Helen Jones. Wife, Ruthann Cruce Jones and daughter, Kelly Kathleen.

Education: Graduated from Pampa High School, 1965. Attended Texas Tech University, 1965-67. Received the Bachelor of Science in Agriculture in May, 1969, and the Master of Science in May, 1973, from Oklahoma State University. Completed the requirements for the Doctor of Philosophy in Animal Nutrition at Oklahoma State University in December, 1975.

Experience: Assistant Research Herdsman for Department of Animal Sciences and Industries, Oklahoma State University, 1969; Herdsman at Vanderburg Swine Company, Pampa, Texas, 1969-71; Graduate Assistant at Oklahoma State University, 1971-75.